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EXPERIMENTS WITH FLAG SMUT OF WHEAT AND THE CAUSAL FUNGUS, UROCYSTIS TRITICI KCKE.[1]

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INTRODUCTION

Urocystis tritici Kcke., the causative organism of flag smut of wheat, though long known to be common in Australia and certain Asiatic countries, was not observed in the United States until May, 1919, when it was discovered by Dr. J. G. Dickson in a wheat field near Granite City, Ill. Because of its reported destructiveness to the wheat crops in other parts of the world, especially Australia, experiments in varietal resistance of wheats were begun in the fall of that year at Granite City. This line of investigation was considered the most expedient at that time because it was known that infestation of soil rather than of seed was the most important factor in the annual occurrence of the disease in Australia. From previous experiments in that country, it had been shown that seed treatment was ineffective in controlling flag smut in infested fields because of infection by viable spores in the soil. Thus, by selecting and sowing varieties which do not become infected in the field, even with artificial inoculation, the disease could be controlled. The results of these and later field experiments relating to varietal resistance and seed treatments have been published recently by Tisdale, Dungan, and Leighty (*15*).[3]

The data here presented are based on greenhouse experiments begun in the autumn of 1919 at Arlington Experiment Farm, Rosslyn, Va. In the first year only preliminary experiments were attempted, such as the testing of 25 varieties of wheat for susceptibility to flag smut and making a detailed study of the diseased plants. In the autumn of 1920 the number of varieties was increased, and as the work progressed and numerous problems presented themselves it was decided to study some of the physiologic aspects of the disease during the following years. The varietal resistance studies, therefore, cover a period of three years, while the other data are based mainly on a year's investigation conducted at Washington, D. C., and Rosslyn, Va., and a second year's study conducted cooperatively with the Missouri Botanical Garden, St. Louis, Mo.

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[2] The writer is indebted to Dr. G. M. Reed, formerly pathologist in charge of cereal smut investigations, under whose direction the varietal resistance experiments were begun; to Dr. W. H. Tisdale, who succeeded Doctor Reed in 1920 and whose aid has been invaluable in the greater part of these investigations; and to Dr. B. M. Duggar, of the Missouri Botanical Garden, St. Louis, Mo., who gave helpful advice concerning the 1922–23 experiments reported in this paper.

Most of the seed used in these experiments was obtained from the agronomic division of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, though that of one or two varieties was obtained from each of the State agricultural experiment stations at Knoxville, Tenn., Urbana, Ill., Manhattan, Kans., Davis, Calif., and Pullman, Wash.

[3] Reference is made by number (italic) to "Literature cited", p. 448–449.

DISTRIBUTION AND ECONOMIC IMPORTANCE

To date (1923) flag smut of wheat has been reported as occurring in the United States, Australia, Japan, China, India, South Africa, Italy, and Spain. In the United States it has been found in Monroe, St. Clair, Madison, Jersey, Macoupin, Greene, Scott, Hancock, and Logan Counties in Illinois; in St. Louis, St. Charles, Warren, Platte, and Buchanan Counties in Missouri; and in Atchison, Leavenworth, Wyandotte, and Miami Counties in Kansas. Field surveys to determine the distribution of flag smut have been made by the State of Illinois and the United States Department of Agriculture each summer for the period from 1919 to 1923. The survey data for each year show that flag smut was found more widely distributed than in the previous year. This increase in the infested area may be due partly to the natural spread of the disease, and partly to the fact that larger areas were surveyed each year, or to either cause alone. From the earlier data it appeared that the disease was spreading rapidly, but evidence from the 1922 and 1923 surveys indicates that it probably has been present for two to several years even in areas reported for the first time to be infested.

During the five years that flag smut has been known to occur in the United States the annual loss due to this disease in the surveyed areas probably has been less than 2 per cent. In parts of some fields, however, 50 per cent of all plants within a given area have been infected. The low average percentage may be due partly to climatic conditions and partly to the fact that most of the time the infested areas have been under State quarantine regulations with regard to disinfection of all grain and threshing machines, the movement of straw, and the sowing of varieties which were found to be fairly resistant to flag smut.

In the literature from Australia, where flag smut was noted first in 1868 (*9*), losses of from 10 to 50 per cent of the wheat crop, due to this disease, commonly have been reported. However, according to information given by R. J. Noble, of Sydney, Australia, the average annual loss due to flag smut alone is about 3 per cent. In some years it is considerably higher, while in others it is practically negligible. Although flag smut has been distributed widely in the eastern wheat-growing area of Australia, it was not known to occur in Western Australia. However, W. M. Carne, who is engaged in plant research in Western Australia, in a letter to Dr. R. J. Haskell, stated that flag smut was recorded as occurring for the first time in that State in October, 1922, but on one farm only.

Butler (*2, p. 171–173, fig. 56*) states that flag smut is confined to the Punjab in India, and that "it has not been reported as causing much damage" in that country.

Hori (*5*) states that flag smut has been known in Japan since 1895 and that within an area of one-fourth acre, in the Province of Kai, in 1898, 100 per cent of the plants were destroyed before flowering time. It has been reported by Hori (*4*)[4] from Gumma and Yamanshi prefectures, and from Higo Province by Yoshino (*17*).[4] Flag smut of wheat is not known to occur in Formosa and Korea. Hori (*6*)[4] further states that this smut is not so well known to the farmers as the other grain smuts, though infrequently it causes considerable damage.

[4] Japanese original translated by Dr. T. Tanaka, formerly botanical assistant and translator, Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

Miyake (*10*) reports the occurrence of flag smut in China and states that it is "very common in Peking and its vicinity."

Putterill (*12*) reports the occurrence of flag smut in South Africa, and states that it probably has been present there for a "number of years," and that "while the total loss up to now may not be considered very great in that district [Marico], yet in some wheat fields lately visited almost half of the crop was found to be affected."

Flag smut of wheat was collected by E. C. Stakman in 1922 at Rieti, Italy, and at Zaragossa, Spain. The economic importance in either country was not determined.

SYMPTOMS OF THE DISEASE

The first indication that a plant in the field is infected with flag smut is the appearance of long grayish-black stripes running parallel with the veins on the upper leaves. At this stage diseased plants in the field appear to be as vigorous as the healthy ones. At a later stage the leaves and upper part of the culms become twisted and curled. Upon close examination of infected plants grown in the greenhouse and field it was found that the young upper leaves showed stripes which were white at their base, becoming whitish gray and finally a lead-gray in color toward the apex of the leaf. Later the epidermis of the leaf ruptures along these stripes and exposes the spores of the fungus in black sooty masses. Plate 1, A, shows some infected leaves with the typical sori. An enlarged portion of an infected leaf also is illustrated (Pl. 1, B). The stripes, or sori, generally are confined to the leaves, although they may occur frequently on the leaf sheaths (Pl. 1, C), occasionally on the upper part of the culm, and still more rarely on the lower glumes and rachis of the wheat head (Pl. 1, D). Plate 1, C, shows the infection occurring on the lower glumes of an underdeveloped head. In many cases all the culms of a diseased plant are infected, although an occasional plant in the greenhouse or field may be found with but one infected culm and the others apparently free from the disease and producing well-filled heads.

In general, the disease seems to stunt the growth of the culms so that no heads are produced. However, in the field in 1922 and 1923 cases were noted where the smutted culms apparently were producing normal heads. These, upon examination, were found in many cases to be sterile, though occasionally a few seeds in each head matured. McAlpine (*9*) has noted cases where small amounts of shriveled grain were produced from infected culms. In a few of these individuals producing sterile heads it was noticed that the infection was confined entirely to the upper part of the culms, there being no infection of any of the leaves of that culm or of the remainder of the plant.

In the greenhouse, plants of some varieties of spring wheat become infected before they begin to tiller. Very susceptible winter varieties also become infected before or at about the time they begin to tiller. Infection may appear at any time from these young stages of development until the plants are mature.

The culms of infected plants grown in the greenhouse do not tend to curl and twist, as commonly is the case in the field. Infected plants were not allowed to mature, which fact might account for the difference in the effect on plants grown in the greenhouse and on those in the field. It was noticed, however, that in certain varieties, such as Hard Federa-

tion, White Federation, and Bobs, of spring habit, the culms often show this character whether inoculated or not. Obviously, then, this can not be regarded as a trustworthy indication of the disease in these particular cases.

During the first year a detailed record was kept for each infected plant of the different varieties. Each plant was examined separately and the number of well-developed culms determined. In addition, the small undeveloped culms were noted. The nodes from which the roots developed, or the crown, were regarded as No. 1, and the others were numbered successively from the base to the apex. The leaves from the first node usually were dead and dry at the time the plants were examined. This also frequently was true of the leaf at the second node, and sometimes also of that at the third, depending upon the maturity of the plant. However, even in this semidry condition, it was possible to determine whether or not these lower leaves were infected. These plants had reached the heading stage, except in a few varieties where the severity of infection necessitated their removal from the greenhouse before that time.

A summary of the data concerning total culm infection for nine varieties in the crop year 1919–20 is given in Table I.

TABLE I.—*Summarized results showing culm infection of wheat plants of different varieties infected with Urocystis tritici and grown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., in 1919–20*

Variety.	Source or C. I. No.	Number of plants—		Percentage of plants with all culms infected.	Culms of partially infected plants.		
		With one to all culms infected.	With all culms infected.		Total number.	Number infected.	Per cent infected.
Baart	1697	16	0	0	113	43	38.1
Bobs	4990	40	24	60.0	122	93	76.2
Cowra No. 3	4119	38	30	78.9	52	37	71.2
Defiance	Calif.	38	5	13.2	268	146	54.5
Fultz	3598	18	0	0	217	114	52.5
Hard Federation	4733	37	29	78.4	51	37	72.5
Harvest Queen	5957	41	28	68.3	142	110	77.5
Do	Ill.	31	17	54.8	143	116	81.1
Propo	1970	18	2	11.1	96	31	32.3
White Federation	4981	40	29	72.5	45	29	64.4

In no variety were all culms infected on all infected plants, though in five varieties over 60 per cent of the plants had all their culms infected, while in two varieties no infected plant had all its culms infected. Of the partially infected plants—namely, those which had both sound and infected culms on the same plant—the percentage of culms infected ranged from 32.3 per cent to 81.1 per cent. In eight of these varieties over 50 per cent of the culms were infected.

In general, every well-developed culm of an infected plant showed infection, the severity being indicated by the number, length, and size of the sori. Rarely a leaf showed no infection, though sori appeared on the leaves above and below. As a rule, however, any culm with the second or third leaf infected would show sori on all other leaves above.

Over 1,600 culms were examined to determine the frequency of the occurrence of sori on the lower leaves of the culms. Sori were found on all the leaves on 16.7 per cent of the infected culms; on the second leaf and all leaves above on 48.7 per cent of the infected culms; on the third leaf and all above on 25.3 per cent; on the fourth and those above on 6.7 per cent; on the fifth and those above on 2.4 per cent; and on the sixth and any above on 0.2 per cent. When infection was noted on the first and second leaves there usually was just a trace of the disease, the infection increasing in severity from the third leaf on up to the apical leaf.

The diseased leaves generally were shorter and narrower than the corresponding leaves of healthy culms. The leaves of sound and of infected culms from the same infected plants of 12 varieties were measured and, even in these cases, the diseased leaves were consistently shorter and narrower than the corresponding leaves on the sound culms of the same plant.

CAUSAL ORGANISM

Flag smut of wheat is caused by a fungus, *Urocystis tritici* Kcke., belonging to the family Tilletiaceae Tul. It was reported by McAlpine (*9*) as occurring in Australia as early as 1868 and determined by Wolff (*16*) in 1873 to be *U. occulta* (Wall.) Rab., the species which occurs on rye (*Secale cereale*). In 1877 Körnicke (7), on examining specimens of flag smut from Australia, separated it from *U. occulta* upon morphological characters alone and named the new species *U. tritici*. However, the name was not adopted, and Saccardo (*13, p. 515*) gave this name as a possible synonym for *U. occulta*, and Sydow and Butler (*14, p. 427*), McAlpine (*8*), and Hori (*5*) still reported this fungus as *U. occulta*.

McAlpine (*9*) conducted cross-inoculation experiments with Urocystis from wheat and rye in 1907 and 1908. He concluded that "the Flag smut of wheat and rye are not mutually infective, and therefore the name given to Flag smut of wheat by Koernicke in 1877, who received specimens from R. Schomburgk in South Australia, should be retained, viz., *Urocystis tritici*."

Urocystis tritici is not known to infect plants of any genus other than Triticum, though several genera have been found to be infected with other species of Urocystis. In 1921, plants of redtop were received which were infected with Urocystis. During the winter of 1921–22 two lots of seed of redtop, Pal timothy, St. John rye (C. I. 130), and Little Club wheat (C. I. 4066) were inoculated, one lot with *U. tritici* and the other with *U. occulta*. These lots of seed were sown on December 1, 1921, in the greenhouse at Rosslyn, Va. There was insufficient spore material from the infected redtop plants to inoculate similar lots of seed. *U. tritici* infected all, or 100 per cent, of the wheat plants, but did not infect any of the rye, timothy, or redtop plants. *U. occulta* infected 7.1 per cent of the rye plants, but did not infect any of the wheat, timothy, or redtop plants.

The spores of flag smut are either solitary or held together in groups of two to several, and the whole completely invested by a layer of small sterile cells (Pl. 2, A). Each spore of a spore ball may germinate, though usually only one or two actually do so (Pl. 2, E, G). However, spore balls with three and even four germinating spores are frequently noted (Pl. 2, H). On germination, the spore sends out a promycelium (Pl. 2, B) with a whorl of one to several sporidia at the apex (Pl. 2, C, D). These sporidia do not become separated from the

promycelium but elongate to form the so-called infection threads (Pl. 2, F *a*, H *d*). Frequently not all of the sporidia elongate (Pl. 2, F *b*), but eventually appear to lose their protoplasm and become hyaline. Fusion of these elongated sporidia and also the production of secondary sporidia (Pl. 2, I *a*) were observed in a very few cases, though in the great majority of instances they merely became elongated. The size and length of the promycelia, sporidia, and infection threads varied with the conditions of germination.

SPORE GERMINATION

The spores of *Urocystis tritici* do not germinate readily or consistently in either distilled or tap water. With spores from material about a month old and kept seven days on soil, McAlpine (*9*) secured limited germination in a water culture after 24 hours incubation. Approximately 40 per cent of the spores of the previous season germinated after being floated on tap water for about four days.

Various solutions and media were tried under different conditions of moisture and temperature by the writer, but though the spores free from any leaf tissue would germinate under one set of conditions at one time, they would not germinate under apparently similar conditions at another. Thus many negative results were obtained. It may be of interest to note those conditions under which fairly high percentages of germination were secured. In 1920 a method was found which since then has given fairly high and uniform results. In these studies spore-laden leaves were powdered and used as a fine dust-like mixture instead of using spores free from leaf tissue. This material was floated on distilled water in a Syracuse watch glass and placed at 18° to 20° C. Some spores germinated at the end of two and three days, although the best germination usually was had on the fourth day. The germination, once started, proceeded very rapidly, and the elongated sporidia, or infection threads, were quite numerous on the fifth day.

Good germination was secured many times in 1921 in tap-water cultures after two to five days incubation. The spores free from leaf tissue, however, did not germinate readily in distilled water, though a few spores sometimes germinated after four or five days. The optimum temperature for germination was 18° to 20° C., though germination at 25° was recorded in one culture and in certain others at 12° to 13°. Satisfactory germination was obtained at 10° after seven to eight days when spores mixed with pulverized leaf tissue were used. However, in infection experiments herein described no infection was obtained at 25°, though it did occur at 6° to 12°.

In December, 1921, fairly good germination took place after three to four days in juice expressed from wheat seedlings and diluted. In some instances germinating seeds were found to stimulate spore germination. In October, 1921, spores which had been dusted on seeds which were placed on soil in a Petri dish in the laboratory germinated freely after two days, while spores from the same source dusted on soil and on the surface of both tap water and a soil solution, did not germinate after four days of incubation.

During the course of these experiments this stimulating effect of germinating seeds on spore germination was observed also in some tests of the viability of spores buried in the soil. For these experiments 14 samples of spores which had been buried under different conditions were

used. The soil and spore mixture constituting each sample was divided into duplicate portions, each of which was placed in a Syracuse watch glass and 3 cc. of tap water added to each. In one portion of each sample five wheat seeds were placed. After four days there was good spore germination in four of these portions in which seeds were germinating, but there was no germination in the duplicate portions without seeds, with the exception of one in which a single germinating spore was found. Also there were a few spores germinating in one other dish in which no seeds had been placed. After seven days there was good germination in four more cultures containing seeds, but there was no increase in germination in the lots without seeds.

Brown (*1*) recorded the stimulating effect of volatile substances arising from plant tissues on the germination of spores of *Botrytis cinerea* and other fungi. Plant distillates and various chemical substances also stimulated germination. Noble's data (*11*) confirm Brown's results on the effect of plant tissues and distillates on spore germination. He found that spores of *Urocystis tritici* "which had been presoaked in water for several days would germinate profusely after the addition of small quantities of wheat seedlings tissue."

VIABILITY OF SPORES

In order to test the viability of spores of various ages, seeds of Harvest Queen (C. I. 5957) were inoculated with dry spores of *Urocystis tritici*, collected in different years and at various places, as given in Table II. The inoculations were made by mixing the seed with the spores in small seed envelopes. The inoculum used in this experiment was obtained from leaves which had been dried and kept at ordinary room temperature under laboratory conditions. The inoculated seed was sown in the greenhouse and infection noted on the plants as they matured. The infection percentages are recorded in Table II.

TABLE II.—*The effect of age of spores of Urocystis tritici, and the locality in which they were produced, on the power to infect Harvest Queen wheat in the greenhouse at Arlington Experiment Farm, in 1921–22*

Row number.	Source of spores.	Year collected.	Wheat plants.		
			Total number.	Number infected.	Per cent infected.
37	Australia [a]	1919	16	16	100
55	Granite City, Ill.	1919	14	9	64.3
46	Rosslyn, Va.	1920	17	9	52.9
64,73	Do.	1921	31	21	67.7
10,19,28	Granite City, Ill.	1921	49	23	46.9

[a] Spores obtained in 1921 from R. J. Noble.

TABLE II shows that the spores produced in 1919 in Australia caused 100 per cent infection, while the others were from 46.9 to 67.7 per cent effective. It may be that the fungus from Australia is more virulent than that obtained in the United States. However, these differences may not be significant, as was shown by the following experiment. Seed of Harvest Queen from the same source as that used in the previous ex-

periment was inoculated with spores collected in the greenhouse at Rosslyn, Va., in 1921, and sown in the same greenhouse bench about 18 days later than the sowings recorded in Table II. Fourteen out of 15 of these plants, or 93.3 per cent, became infected. The seed and spores were from the same source as those used in rows 64 and 73, given in Table II, in which but 67.7 per cent of the plants became infected. Therefore it is reasonable to expect that had the conditions at the time of sowing been similarly varied for all the spore lots used in the inoculations noted in Table II, different percentages of infection might have been obtained. However, the results serve to indicate that the infective power of spores is not destroyed even though they are stored two or three years under laboratory conditions.

In the spring of 1923 germination tests were made with spores from the same collections listed in Table II. In all instances good germination was obtained. Thus it has been shown that the spores collected in Australia and Illinois in 1919 were viable after having been kept for four years in the laboratory.

In order to test the infective power of fresh spores, seed of Harvest Queen was inoculated with spores from infected green leaves collected on January 5, 1922, and sown in clean soil on January 6, 1922, together with one uninoculated row and one row inoculated with dry spores of the previous season for controls. The fresh spores produced an infection of 5.7 per cent and the dry spores 47 per cent, while no infection developed in the uninoculated row. Again in March, 1923, 30 seeds each of Harvest Queen, Hard Federation, and Little Club were inoculated with spores which had been collected the same day, and then were sown in three 10-inch pots containing garden soil autoclaved for 15 minutes at 15 pounds pressure. The pots were set in the soil on a greenhouse bench in order to lessen drying out and to prevent rapid temperature changes. Two plants of Hard Federation, or 6.7 per cent, became infected, but no infection developed in Harvest Queen or Little Club. Thus it was shown that wheat plants can become infected by fresh spores, though the percentages of such infection may be quite small.

OVERWINTERING OF SPORES

It having been shown that soil infestation was an important factor in the dissemination of flag smut, and one which made seed treatment partially ineffective, it was thought desirable to secure experimental data on the overwintering of the spores in the soil. On such information recommendations could be based for such practices in soil management as might control the disease.

The following experiment was conducted to determine the ability of spores to overwinter in the soil at Granite City, Ill., in the flag-smut area. Forty-two small cylindrical wire baskets about 1 inch in diameter and 3 inches long were filled with the various mixtures of different soils, diseased leaves, and spores of the previous season, as given in Table III. Besides the native black alluvium of Granite City, there were used sandy loam and basaltic soils from Madison, Wis., and Pullman, Wash., respectively. These baskets were buried in the ground at different depths on October 6, 1921.

One-third of the baskets were dug up on November 1, one-third on November 28, and the final third on April 10, 1922, and the contents sent to the laboratory in small glass vials. A soil suspension was made from each, using about 30 cc. of tap water. The spores in part were

recovered from the soil by fractional centrifuging. The heavier soil particles with many spores were centrifuged out first and then a layer, consisting of many spores and the lighter soil particles, was obtained. To test spore germination, this layer was pipetted off into a Syracuse watch glass, tap water added, and the whole placed in an incubator at 18° C. But it had been found that flag-smut spores often fail to germinate in cultures, although spores from the same source produced abundant infection in susceptible plants inoculated with them. So it was decided to grow seedlings in the heavier residue to determine the viability of these overwintered spores by their infective power. Five seeds of wheat were sown in each soil and spore residuum and allowed to grow in the same chamber with the spore germination tests. Harvest Queen was sown in the series dug up on November 1 and November 28, while in the series dug on April 10, 1922, both Harvest Queen and Bobs were sown.

When these seeds had germinated and each plant had one well-developed leaf, they were transplanted to clean soil in the greenhouse. Infection in the earlier sowings was first observed in January, and the final results were recorded in June. These data, together with the results of the spore germination tests, are recorded in Table III.

TABLE III.—*Viability and infective power of spores of Urocystis tritici buried for varying periods in different types of soil at Granite City, Ill., on October 6, 1921*

Soil and inoculum.	Depth of burial.	Dates baskets were dug from soil.						
		Nov. 1, 1921 (Series 1).		Nov. 28, 1921 (Series 2).		Apr. 10, 1922 (Series 3).		
		Spore germination.	Infection on Harvest Queen.	Spore germination.	Infection on Harvest Queen.	Spore germination.	Infection on Harvest Queen.	Infection on Bobs.
	Inches.		*Per ct.*		*Per ct.*		*Per ct.*	*Per ct.*
Black alluvial soil plus spores	0– 2	–	40.0	+	0	–	0	0
Do	2– 4	–	0	+	0	–	0	0
Do	4– 6	–	75.0	–	0	–	0	0
Do	6– 8	–	75.0	–	0	–	0	0
Do	8–10	–	50.0	–	0	–	0	0
Black alluvial soil plus infected leaves	0– 2	+	0	+	0	–	0	0
Do	2– 4	–	0	+	0	–	0	20
Do	4– 6	+	40.0	+	0	–	0	0
Do	6– 8	+	0	–	0	–	0	20
Do	8–10	–	0	+	0	+	0	0
Basaltic soil plus spores	4– 5	–	33.3	–	0	–	0	0
Basaltic soil plus leaves	4– 5	–	40.0	+	20	–	0	0
Sandy loam plus spores	4– 5	+	30.0	–	0	–	0	0
Sandy loam plus leaves	4– 5	–	0	–	0	–	0	0

The foregoing results seem to indicate that the infective power of the spores was markedly decreased during the time between the removal of the first and second series, or between November 1 and 28. The spore germinations show a slight increase, due probably to a difference in the method of germination. The two cases of infection obtained in Series 3 from spores buried over five months were noted on Bobs, a very susceptible spring wheat which had not been used for the earlier tests.

Another series of experiments on the overwintering of spores was conducted cooperatively with the Missouri Botanical Garden during the winter of 1922–23. Diseased leaves had been collected in 1922 from plants grown in the field at Granite City, Ill., and in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. These leaves were ground in a food chopper, mixed with two parts of soil in a Mason jar, and placed in small, 2-inch flowerpots on November 24, 1922. These pots were divided into two lots and buried in the soil so that the tops of one lot were 1 inch below the surface of the ground and those of the other 5 inches below. The height of the pots being 2.5 inches, the spore mixtures in each pot of the first lot were from 1 to 3.5 inches and those in the other from 5 to 7.5 inches beneath the soil surface. The two depths at which the pots were buried are recorded in Table IV as 2 and 6 inches, respectively. In addition, six pots, similarly filled, were watered and kept in the greenhouse for 4, 8, and 12 days. At the end of each of these three periods, two pots were buried, one at each depth recorded above. As the viability of the spores is considered to be decreased during long intervals of wetness, the late sowings of spores which had been watered for 4, 8, and 12 days before burial were intended to simulate to a slight extent the periods of fall rains. Thus, if the spores had been in the soil through late summer and early fall, their viability might have been lessened by the time these experiments were begun. As a check on the viability of the spores a part of the soil and spore mixture was kept in the Mason jar until the first pots were dug on January 4, 1923.

At various intervals of about two weeks, some of the pots in each series were taken into the greenhouse. Seed of Little Club was sown in the pots and allowed to grow. The soil temperatures for the different sowings were not the same, the temperatures for March and April being considerably higher than those for January and February. After the resulting seedlings had produced one or two strong leaves, they were transplanted in clean soil in the benches. The pot buried on November 24, with the spores 2 inches below the surface, had its contents destroyed by mice after it was dug up on January 4 and wheat seed sown. Also the seed sown on January 4 in the pot buried 6 inches below the soil surface on December 6 and brought in on January 4 was destroyed by mice. Additional seed was resown in this pot on January 12, 1923. The infection results are given in Table IV.

From Table IV it seems that watering the spores and keeping them in the greenhouse for 4, 8, and 12 days before they were buried in the soil did not diminish their viability. Indeed, four of the rows showed 100 per cent infection, which was slightly higher than that of the control. The spores showed a marked decrease in viability about February 3. The coldest weather of the year was during the two weeks following this time, the minimum temperature recorded being about 1° F. By February 18, the ground had begun to thaw and the pot at the 2-inch depth was brought in, though the one at the 6-inch depth could not be dug from the ground until February 22. There was an infection of 11.1 per cent in the plants grown in the pot buried at the 6-inch depth and brought in on March 16. There was no infection in the plants sown in pots removed from the soil on April 14, but the plants did not grow well because of the high air and soil temperatures in the greenhouse. However, as previously stated, there was infection in the plants sown in April, 1922, in the infested soil which had been removed from the field at Granite City, Ill., on April 10, 1922.

TABLE IV.—*Infections of Little Club wheat produced by spores of Urocystis tritici buried at different depths and for varying periods during the winter of 1922–23 at the Missouri Botanical Garden, St. Louis, Mo.*[a]

Average depth spores were buried.	Date when spores were—		Total number of plants.	Infected plants.	
	Buried.	Exhumed.		Number.	Per cent.
Inches.					
Control	.....	.....	22	21	95.5
2	Nov. 28	Jan. 4	28	25	89.3
6	....do.....	....do.....	25	25	100
2	Dec. 2	....do.....	19	19	100
6	....do.....	....do.....	27	27	100
2	Dec. 6	....do.....	8	8	100
6	....do.....	....do.....	24	20	83.3
6	Nov. 24	....do.....	25	24	96.0
2	....do.....	Feb. 3	24	6	25.0
6	....do.....	....do.....	23	12	52.2
2	....do.....	Feb. 18	26	5	19.2
6	....do.....	Feb. 22	22	6	27.3
2	....do.....	Mar. 1	18	3	16.7
6	....do.....	....do.....	20	0	0
2	....do.....	Mar. 16	18	0	0
6	....do.....	....do.....	18	2	11.1
2	....do.....	Apr. 14	18	0	0
6	....do.....	....do.....	18	0	0

[a] Experiments in cooperation with the Missouri Botanical Garden.

On October 5, 1921, a plot of land about 10 feet square, near the experimental field at Granite City, Ill., was inoculated thoroughly with flag-smut spores by spading under heavily infected wheat straw. On October 12, 1922, over one year later, clean seed of Harvest Queen wheat was sown in this plot. An uninoculated control plot of equal size, located some distance away, also was sown with uninfested seed of the same variety. In May, 1923, there were nine infected plants, or 2.4 per cent, in a total of 377 in the inoculated plot. None of the 370 plants in the control plot was infected.

Thus it has been shown that, though there is a decrease in viability, the spores are able to survive a winter and subsequent summer in the flag-smut area near St. Louis, Mo. They not only are capable of germination, but actually can infect wheat plants.

INFECTION

Regarding the infection of wheat by *Urocystis tritici*, McAlpine (*9*) states: "Infection occurs in the seedling stage, also when the young shoots are being formed, but not when the plant is above ground, . . ." He proved that infection did occur in the seedling stage, but based his conclusions concerning the infection of new shoots as they are formed during tillering, on some data given by Hecke (*3*). The latter cut back to the collar shoots of sound plants of the perennial rye (*Secale montanum*) in the autumn. He then dusted spores of *Urocystis occulta* on the exposed collar and covered the plant with manure containing the same kind of spores. In the spring the shoots produced were infected. Discussing Hecke's data, McAlpine says: "But a fresh light has been thrown on the infection of rye, and the same probably applies to wheat. By the experiments of Hecke . . . it was there shown that the spore has not only

the one chance of infecting the primary or terminal bud, but also the numerous chances of infecting the lateral buds produced beneath the surface of the soil and growing out into fresh stalks. There is not only seedling infection, but shoot infection, and it is decidedly to the advantage of the parasite to multiply the points of attack as much as possible."

If it be true that new shoots as well as the plumule are liable to infection before emerging from the soil, then factors which would hasten growth through the soil would shorten the infection period and, therefore, would lessen the chance of infection. Soil moisture and temperature would play an important part by influencing the germination both of the wheat seed and of the smut spores.

Experiments were conducted to determine the relation, if any, between infection and date of sowing, temperature, stage of growth prior to inoculation, and tillering. These experiments are discussed separately. In addition, experiments were conducted to determine the effects of cutting back inoculated plants at different periods of growth on the appearance of flag-smut infection.

DATE OF SOWING AS RELATED TO INFECTION

The following experiment was conducted cooperatively with the Missouri Botanical Garden, to determine if any relation existed between the date of sowing inoculated seed and the amount of infection produced. Harvest Queen was used throughout this experiment. Eleven sets of 125 selected seeds each were inoculated and sown in rod rows at three-day or four-day intervals from October 17 to November 21, 1922. The rows were 1.5 feet apart, and the seeds were spaced at intervals of a little less than 2 inches. All inoculations were made simultaneously with dry, ground spore material of the previous season. The vials of inoculated seed for the later sowings were kept in a cool, dry place in order to maintain the viability of both the spores and seed. Infection was first observed on April 28, 1923, on some of the plants of the first sowings. The dates of the various sowings are given in Table V, together with the total number of plants, number of infected plants, and the percentage of infection for each date of sowing.

TABLE V.—*Percentages of infection of Harvest Queen wheat by Urocystis tritici, as influenced by date of seeding from October 17 to November 21, 1922, in the Missouri Botanical Garden, St. Louis, Mo.*[a]

Date sown.	Plants.		
	Total number.	Number infected.	Per cent infected.
Oct. 17	31	19	61.3
Oct. 20	48	24	50.0
Oct. 24	37	14	37.8
Oct. 27	26	6	23.1
Oct. 31	24	5	20.8
Nov. 3	24	7	29.2
Nov. 7	50	7	14.0
Nov. 10	79	19	24.1
Nov. 14	73	0	0
Nov. 17	72	0	0
Nov. 21	69	0	0

[a] Experiments in cooperation with the Missouri Botanical Garden.

The percentage of infection decreased from 61.3 per cent in the sowing of October 17 to 20.8 per cent in the sowing of October 31. There was no infection in the rows sown on or after November 14. These variations in the percentage of infection were due probably to soil temperature and moisture conditions, which inhibited the germination of the spores at the time the wheat was germinating.

TEMPERATURE AS RELATED TO INFECTION

A series of experiments was arranged in order to determine the range of temperature within which actual infection could occur. On December 1, 1921, 40 seeds of each of five susceptible varieties of wheat (Bobs, Hard Federation, Harvest Queen, Little Club, and White Federation) were inoculated heavily with flag-smut spores by moistening the seeds and rolling them in dry spores. The seeds were then incubated between moist blotting papers in the manner prescribed for standard seed-germination tests. Through the kindness of W. L. Goss, of the Seed Testing Laboratory of the Bureau of Plant Industry, germination chambers were secured in which the temperature was controlled within narrow limits by electric thermostats and in which a humidity of practically 100 per cent was maintained. Recording maximum and minimum thermometers were placed in each chamber. The three chambers used had average temperatures of 17.8° C., 22.3°, and 25.8°, with fluctuations between 17° and 19°, 21.5° and 23.5°, and 25° and 26.6°, respectively, during the time the seeds were in the chambers. A fourth lot of seeds of the same varieties was placed in an ice box, the temperature of which ranged from 6° to 12° during the 30 days of the experiment.

When the seedlings in each chamber had reached the stage where the first leaf had pushed through the coleoptile, they were washed thoroughly with the aid of a brush until no spores were visible on the seeds. They were then transplanted to clean soil in the greenhouse, in December, 1921, and allowed to grow until flag-smut lesions appeared or, if the plants did not become infected, until maturity. The infection percentages obtained at each temperature are given in Table VI.

TABLE VI.—*The effect of temperature on infection of five varieties of wheat by Urocystis tritici when inoculated seed was germinated on blotters in temperature chambers, then washed and transplanted in greenhouse soil in December, 1921*

Variety.	Average temperature.											
	9° C.			17.8° C.			22.3° C.			25.8° C.		
	Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.	
		No.	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*
Bobs	30	5	16.7	25	3	12.0	19	6	31.6	27	0	0
Hard Federation	29	0	0	19	0	0	16	7	43.8	26	0	0
Harvest Queen	23	0	0	32	6	18.8	28	9	32.1	30	0	0
Little Club	23	0	0	23	1	4.3	27	6	22.2	31	0	0
White Federation	.....	.....	.....	18	0	0	14	2	14.3	14	0	0
All varieties	105	5	4.8	117	10	8.5	104	30	28.8	128	0	0

The data show that only one variety, Bobs, became infected at 6° to 12° C. and that the highest percentage infection for each variety occurred at 21.5° to 23.5°. The fact that no infection developed in any of the five varieties when grown at 25° to 26.6° is probably due either to the accelerated growth of the wheat plants at this higher temperature, which enabled them to escape infection, or to the inhibition of spore germination, or to both effects.

Even at the optimum temperature all of the percentages of infection are relatively low for such susceptible varieties. In the varietal resistance experiments for 1921–22, herein described, in which the inoculated seed was sown in the soil, the percentages of infection in these same varieties were 86.7 for Bobs, 80.8 for Hard Federation, 88.5 for Harvest Queen, 97.8 for Little Club, and 97.4 for White Federation.

A preliminary experiment on the effect of controlled soil temperatures on infection was conducted by W. H. Tisdale and R. W. Leukel in the greenhouse at Arlington Experiment Farm in the spring of 1923. An equal amount of soil was placed in each of 12 galvanized cans. Two cans were set in water in each of six tanks held at 10°, 15°, 20°, 24°, 28°, and 32° C., respectively. Twenty seeds of Bobs wheat were sown in each can on March 26. The seeds were spaced at regular intervals in a circle, 1.5 inches from the border of the can, and covered with about 1 inch of soil. No smut developed in any of the cans except the ones in which the soil temperature was held at 20°. In these cans 5, or 12.5 per cent, of the 40 plants became infected with flag smut.

STAGE OF GROWTH AS RELATED TO INFECTION

In the case of seedling infection it is stated by McAlpine (9) that infection occurs before the plant emerges from the soil. Soon after emergence the coleoptile, or sheath, which completely invests the plumule, is broken, and the latter appears. In order to determine the stages of seedling growth at which infection could occur, the following experiments were conducted. Seed of Bobs, Hard Federation, Harvest Queen, Little Club, and White Federation, the same varieties used in the temperature experiments, were placed between moist blotters, at 18° to 20° C. in a seed-germination chamber on December 6, 1921. When these seeds were at three definite stages of germination—namely, (1) testa, or seed coat, just broken; (2) plumule about 1 to 1.5 cm. long, and three strong roots developed; and (3) coleoptile just broken, those in each stage were divided into two lots. In addition, dry, ungerminated seed was divided into two lots. Both lots were inoculated and placed in clean soil in the greenhouse December 9 to 16, 1921. One lot of each was inoculated by rolling the seed or seedling in dry spores and the other by immersing in a spore suspension. This suspension consisted of spores soaked in tap water for at least two days. The spores in suspension were not necessarily germinating. It was thought that if the time between the transplanting and emergence of the seedlings was very short the dry spores might not have time to germinate and infect the seedlings before the latter came through the ground. On the other hand, if the spores were thoroughly soaked they could germinate in a very short time. Unfortunately, the emergence dates for these seedlings were not recorded, although these data would have been valuable in correlating rate of growth and infection. Table VII contains the results for the two lots of each variety inoculated with the soaked and dry spores, respectively, at the three stages of growth described above.

TABLE VII.—*Results of inoculating wheat seedlings at three definite stages of growth with both soaked and dry spores of Urocystis tritici and planting them in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., December 9 to 16, 1921*

Variety.	Seedlings inoculated with—						Average percentage of infection including both lots.
	Lot 1. Soaked spores.			Lot 2. Dry spores.			
	Total plants.	Infected plants.		Total plants.	Infected plants.		
		Number.	*Per cent.*		*Number.*	*Per cent.*	
SEED COAT JUST BROKEN.							
Bobs	36	4	11.1	35	17	48.6	29.6
Hard Federation	34	5	14.7	33	16	48.5	31.3
Harvest Queen	32	6	18.8	34	6	17.6	18.2
Little Club	31	20	64.5	28	10	35.7	50.8
White Federation	33	20	60.6	30	23	76.7	68.3
PLUMULE AND THREE ROOTS DEVELOPED.							
Bobs	35	5	14.3	34	12	35.3	24.6
Hard Federation	35	9	25.7	36	11	30.6	28.2
Harvest Queen	34	10	29.4	33	4	12.1	20.9
Little Club	30	25	83.3	31	18	58.1	70.5
White Federation	29	18	62.1	29	16	55.2	58.6
COLEOPTILE BROKEN.							
Bobs	35	0	0	36	0	0	0
Hard Federation	36	0	0	36	0	0	0
Harvest Queen	35	0	0	34	0	0	0
Little Club	32	0	0	33	0	0	0
White Federation	.....	.....	.....	.....	.....	.....	.....
CONTROL: DRY UNGERMINATED SEED.							
Bobs	17	8	47.1	.....	.....	.....	47.1
Hard Federation	17	8	47.1	.....	.....	.....	47.1
Harvest Queen	15	7	46.7	17	9	52.9	50.0
Little Club	8	8	100	8	6	75.0	87.5
White Federation	7	5	71.4	7	5	71.4	71.4

Abundant infection occurred in the plants inoculated at the first two stages, but in no case was there infection of any plant which was inoculated after the coleoptile was broken. The highest percentage of infection in the three-root stage was 83.3 per cent in Little Club, and in the stage where the seed coat was just broken there was 76.7 per cent in White Federation. There apparently was no relation between the condition of the inoculum at time of application and the percentage of infection produced in each case. In some instances where the inoculations were made at the same stage of growth the dry inoculum produced a higher percentage of infection, while in others the spore suspension produced the higher percentage. Four of five varieties had higher percentages of infection when inoculated with dry spores at the time the seed coat was just broken than when the inoculations were made at the three-root stage. All of the varieties inoculated with the spore suspension had higher percentages of infection when they were inoculated at the three-root stage than when the seed coat was just broken. In the varietal-resistance experiments herein described infections of 100 per cent were

recorded in a few varieties. Obviously, in these cases, inoculating the seeds with dry spores gave as high a percentage as could have been obtained if the seeds had been inoculated with soaked or even germinating spores. These results would indicate that the period favorable for infection is before the seedling comes through the ground, or before the coleoptile is broken.

In order to test the possibility of infection during the tillering stage, when new shoots are produced from the crown of the plant, the following experiments were conducted: One lot of smut-free seed of Harvest Queen, Little Club, and White Federation, the same varieties used in the foregoing experiments, was sown in clean soil on November 28, 1921. A similar lot was inoculated with dry spores of the previous season and sown on the same day. Three rows of each variety in the two lots were sown. On December 13, when the plants in both lots had developed one strong leaf and a small second one, they were removed from the soil. Only two rows of each variety in the two lots were removed. For a control the third row in each instance was not disturbed. The uninoculated plants then were inoculated by rolling the roots and lower part of the stem in dry spores and were transplanted to the rows in which the inoculated seed had been sown. The inoculated plants were washed free from all soil particles and the old attached seed washed and brushed until all traces of the spores were removed. They then were planted in the clean soil where the uninoculated seed had been sown. Thus, there was an exact reversal of position. Both sets also were inoculated, one on the original seed and the other when the plants were about two weeks old. The results are given in Table VIII.

TABLE VIII.—*Infection of three varieties of wheat by Urocystis tritici after inoculating seed and seedlings in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., from November 28 to December 13, 1921*

Variety.	Control.						Uninoculated seed; seedlings inoculated and transplanted to infested soil.			Inoculated seed; seedlings washed and transplanted to clean soil.		
	Clean seed in clean soil.			Inoculated seed in clean soil.								
	Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.	
		No.	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*
Harvest Queen	16	0	0	18	14	77.8	30	1	3.3	34	30	88.2
White Federation	17	0	0	14	11	78.6	30	0	0	20	18	90.0
Little Club	17	0	0	14	14	100	35	1	2.9	32	30	93.8
All varieties	50	0	0	46	39	84.8	95	2	2.1	86	78	90.7

There was one plant each of Harvest Queen and Little Club infected in the lot which was inoculated when the plants were about two weeks old, but no infection appeared in White Federation. No other infection was noted on plants which were inoculated after the seedlings had emerged. There was 88.2 per cent or more of infection in each variety which was inoculated at the time the seeds were sown. From these results and from the data obtained in the previous experiment where

no infection occurred in 277 plants when inoculation was made after the coleoptile was broken, it would seem that in the greenhouse, at least, shoot infection was almost negligible.

RELATION OF CUTTING BACK INOCULATED PLANTS TO DEVELOPMENT OF SMUT

In addition to the possible infection of shoots during the process of tillering, there is another type of shoot infection which evidently comes from within the plant and is the result of the original seedling infection.

In the spring of 1921 inoculated plants of Bunyip, Cedar, and Comeback, grown in the greenhouse at Arlington Experiment Farm, apparently were free from the disease. Each plant had about four culms, all of which were yellow and well headed at the time of note taking. As these varieties had been practically free from the disease in the previous season, and as other infected varieties needed immediate attention, they were allowed to mature. However, the plants were watered each day, and small secondary shoots soon began to develop at the base of the mature culms. On later examination several of these shoots were found to be infected, even though the mature plants had shown no infection. The occurrence of the disease in these small, secondary shoots, while the headed culms of the same plants remained smut-free, possibly may be explained in two different ways. First, it may be assumed, as McAlpine has suggested, that they were infected by spores present in the soil at the time of emergence; or, second, that they were infected from mycelia already in the plant, which, for some reason, had not been able to form spores in the older leaf tissue.

Greenhouse experiments were conducted in 1921–22 to determine the possible source of infection in such secondary shoots and to study the effect of cutting back the plants at various stages of growth on the production of diseased secondary shoots. The results of the experiments in which seedlings had been inoculated immediately following rupture of the coleoptile and later, during tillering, have already been given. There was no infection of any shoot when the inoculations were made just after the coleoptile had been broken. Two plants out of a total of 95 were infected when inoculations were made after the seedlings were about two weeks old.

Again, 50 uninoculated seedlings of Harvest Queen were cut to the level of the ground immediately after the coleoptile became ruptured. The cut surface of each seedling and the soil around it were heavily inoculated with flag-smut spores. No infection developed in any of these plants. In later experiments no new cases of infection have been noted where inoculation of the soil and plants was delayed until the latter had emerged from the soil.

None of the soil in which the cut-back plants were grown was inoculated with flag-smut spores. The spores were sown with the seed and, therefore, were about 2 inches under ground. Thus there were no spores near the surface of the soil excepting those which might have been carried up through the soil by the plumule during its growth.

The crowns of wheat plants grown in the greenhouse were practically on the surface of the soil, so that often the secondary shoots never came in contact with the soil. The shoots arising from the base of the culms often were pressed closely to the culms. In cases of this kind the basal leaf sheaths of the culm incased both the secondary shoots and the lower

part of the culms until the shoots were from 1 to 3 inches high. The secondary shoots arose from the crown or from the first, second, and third nodes above the ground. Infection often was noted on shoots which arose from these points of origin. Plate 3, A, shows a cut-back plant with an infected shoot which arose from the second node, or the first node above ground, of a culm which had been cut. This infected shoot from the second node is enlarged in Plate 3, B.

From the fact that the infected shoots often arise from the nodes above ground, where the chances for infection are very few, and from the difficulty encountered in producing infection where inoculations were made after the plants were above ground, it would seem that the smut appearing on the secondary shoots arose from hyphae already inside the tissues of the host plant.

Seventeen winter varieties and 11 spring varieties were used in experiments to study the effect of cutting back wheat plants on the appearance of infection. These varieties were resistant, slightly susceptible, and, in a few instances, quite susceptible in previous varietal experiments. A susceptible spring and a susceptible winter variety, Bobs and Harvest Queen, were used as controls for infection. Four rows of each veriety were sown 6 inches apart, placing 18 inoculated seeds 2 inches apart in each row. The plants in row 1 of each variety were cut back to about 1 inch in height when they had two leaves and were two to three weeks old. The plants of the second row of each variety were cut back to 2 to 3 inches in height when they had two or three small culms, or when infection was showing in the susceptible varieties used as controls. Plants in the third rows were cut back when beginning to head, and those in the fourth rows were left uncut and used as controls on the plants which were cut back. These different series of cut-back plants are hereafter referred to as rows 1, 2, 3, and 4.

The appearance of smut lesions was watched for closely, and all infected plants removed as soon as observed, so that in each instance only the smut-free plants were cut back. Of the 11 spring varieties treated in this manner 3 showed no infection and 2 an infection of 1.6 and 5.4 per cent, respectively, but which did not seem to be influenced by cutting back the plants. However, 5 varieties developed smut in the cut plants either earlier or in a greater percentage than in the plants which were not cut back. Of the 17 winter varieties, 10 showed no smut, while 4 showed only one or two infected plants. Thus in only 3 winter varieties was the development of smut influenced to any extent by cutting back the plants. The percentage of plants infected in each row of the 8 different varieties which became infected is given in Table IX.

There was no uniform plant response from each variety at each stage of development when cut back. In all varieties where the plants were cut back in the two-leaf stage, there was a greater percentage of infection than in the control rows which were not cut back. There was considerable variation in the percentage of infection in rows 2 and 3. Moreover, it was observed that clipping the culms not only influenced the percentage of infection, but, in many instances, the interval between sowing and appearance of smut was considerably shortened.

Clipping the plants after they had begun to head was the most favorable with regard to both the percentage and the appearance of infection in Bunyip. Small, secondary shoots at once began to develop in row 3, and the lesions appeared 17 days earlier in this row than in rows 2 and

4, and 34 days earlier than in row 1. Row 3 had a final infection percentage of 58.3 in comparison with 31.3 per cent in the control row. There was one plant in row 1 which was cut back twice before infection was noted—once in the two-leaf stage, and again after the plant had headed. Finally a small secondary infected shoot was put out from the first node above ground.

TABLE IX.—*Percentages of infected plants in eight wheat varieties inoculated with Urocystis tritici, and cut back at various stages of growth, in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., in 1921–22*

Variety.	C. I. No.	Row number and stage when cut back.			Control.
		1	2	3	4
		Two-leaf.	Time of normal infection.	Heading.	Not cut.
Bunyip	5012	40.0	40.0	58.3	31.3
Comeback	4991	90.9	72.7	84.6	[a] 20.0
Florence	5129	50.0	50.0	60.0	31.6
Sonora	4293	36.3	23.0	22.2	25.0
Pacific Bluestem	3019–3	83.3	43.7	42.8	33.3
Flint	6612	54.5	26.6	15.1	41.2
Fultz	3598	40.0	77.7	13.3	18.8
Jones Fife	5608	64.2	44.4	46.6	42.9

[a] Normal infection. An additional 73.3 per cent of the plants showed infection in small secondary shoots which appeared after the first culms had matured.

A striking difference in infection percentages was shown in the various rows of Comeback. It will be noticed from figure 1 that by the time there was 72.7 per cent of infection in row 1 there was 15.3 per cent in row 3, 9 per cent in row 2, and 20 per cent in row 4. None of the plants in rows 2, 3, and 4 had been cut back at this time. After all infected plants had been removed from the four rows the remaining sound plants in row 2 were cut the same day. Row 3 was cut back about one month later. Small shoots soon were produced at the crown and nodes, and many of these were infected with flag-smut sori. The dotted line in row 4, or the control, represents the infections which appeared in the small secondary shoots which were produced after the culms were practically mature.

Row 1, sown to Florence, produced 18.4 per cent more smut than the control, and the period of smut maturation was reduced by 30 days. Sixty per cent of the plants in row 3 were infected. This was the maximum for this variety, and exceeded by 28.4 per cent the infection recorded for the control row.

The final percentages of infection for Sonora did not show great differences. Row 1 produced 36.3 per cent, or 11.3 per cent more infection than developed in the control row. Row 2 contained 23.0 per cent of infection in comparison with 25.0 per cent in the control, but it should be noted that the total percentage of smut recorded for row 2 had developed 22 days before infection appeared in row 4, and 73 days before the last infected plant was noted in row 4. Although row 1 showed the highest percentage of infection, no lesions appeared in row 1 until 13 days after

they were first noted in the control row. This late appearance of infection in row 1 of Sonora was the same as in row 1 of Bunyip.

In Pacific Bluestem the control row showed 33.3 per cent of smut, while rows 1, 2, and 3 showed 83.3, 43.7, and 42.8 per cent, respectively. Moreover, the total 83.3 per cent of smutted plants in row 1 appeared 35 days before infection was first observed in the control row.

Row 1 of Flint showed 54.5 per cent of the plants infected in comparison with 41.2 per cent in the control and with 26.6 and 15.1 per cent in rows 2 and 3, respectively. The total 54.5 per cent of smutted plants in row 1 appeared 113 days before infection was first observed in the control row.

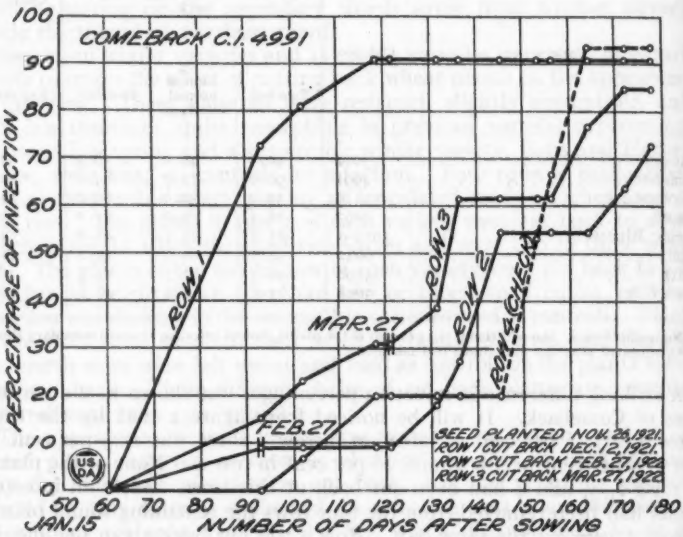

FIG. 1.—Graph showing the time of appearance of *Urocystis tritici* and the percentage of infection on plants of Comeback wheat cut back at different stages of growth while growing in the greenhouse at Roslyn, Va., 1921–22.

Fultz showed 40 and 77.7 per cent, respectively, in rows 1 and 2 in comparison with 18.8 per cent in the control row, and the final infections were noted in the first two rows more than 30 days before smut was first observed in the control row. Smut lesions appeared about the same time in rows 1 and 2, but did not appear in rows 3 and 4 until over 30 days after their first appearance in rows 1 and 2.

In Jones Fife the sori appeared about the same time in the first three rows and over 30 days before they appeared in the control row. Row 1 had 21.3 per cent more smutted plants than the control row. Rows 2 and 3 showed only slightly higher percentages of infection than the control, though the appearance of the disease was noted in each row more than 30 days before it appeared in the control row.

It is significant to note that in many cases infection can be produced earlier and in greater percentage by cutting back the plants. As previously noted, the same effect was produced in Comeback by continued

watering after maturity. Row 4 of this variety, which had not been cut, showed only 20 per cent of the plants infected at the time the culms became yellow and were maturing. By continuous watering, small secondary shoots were produced, which probably were comparable to those produced when the plants were cut back. There was a total infection of 93.3 per cent of the plants, or an increase of 73.3 per cent, that was the result of the appearance of smut sori in these small secondary shoots. In view of these facts it is possible that many apparently quite resistant varieties are not suitable for breeding for resistance to flag smut. However, varieties showing this tendency to develop smut only in secondary shoots undoubtedly evidence a certain degree of resistance. Resistance was shown also by other varieties where the only infection on a whole plant consisted of a single very minute sorus. In one case a small lesion was found on a lower glume of a wheat head, thus indicating that the fungus had kept pace with the growth of the plant but was not able to sporulate extensively. In other instances there were only two or three minute sori on one leaf of an entire plant. This would indicate that the fungus was in the plant tissue even though the spores were not produced freely. As sporulation is the only superficial evidence of flag-smut infection, cytological investigation might determine the presence or absence of mycelia in the leaves and culms of plants which apparently are free from the disease. It also might indicate whether or not the mycelia generally passed beyond the lower nodes of the culms.

VARIETAL RESISTANCE

Experiments in varietal resistance were conducted during three winters, 1919, 1920, and 1921, in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. In addition to the commercial winter wheats grown in Illinois, several other varieties of spring and winter wheats were included. Twenty-one varieties were tested for three years, while others were tested for one or two years. All inoculations were made by placing the seed and a sufficient quantity of dry ground leaves containing spore material in an envelope and shaking until the seed was covered with spores. The inoculated seeds were sown 2 inches apart, and about 2 inches deep, in rows 6 inches apart in the greenhouse benches. In 1920 and 1921 small quantities of spore material also were placed on each inoculated seed before covering with soil. The soil was moist and in good tilth, and the air tenperature was maintained approximately at 55°F. The data were obtained when the plants were beginning to head, or heading, except in cases of severe infection, which necessitated the removal of the plants before that time. Infection was noted for both culms and plants, except in 1922, when only the plant infections were recorded. The results for the three years are given in Table X.

Of the 21 varieties which were tested for three years only Fulcaster, Poole, Red May, Red Rock, Early Defiance, and Galgalos were free from flag-smut infection during that time. Of 17 additional varieties tested for two years Illini Chief, Treadwell, Turkey, and Peliss were free from infection. The highest percentages of smutted plants recorded for the most susceptible varieties in any one year were 78.9 per cent for Fultz, 93.1 for Harvest Queen, 86.7 for Jones Fife, 66.1 for Purplestraw, 72.9 for Red Wave, 98.4 for Bobs, 93.3 for Comeback, 92.7 for Cowra No. 3, 88.4 for Defiance, 98.2 for Hard Federation, and 100 per cent for Little Club and White Federation.

TABLE X.—*Infection of wheat, spelt, and emmer by Urocystis tritici produced by inoculating the seed with dry spores and sowing in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., during one or more of the three years, 1919, 1920, and 1921*

Crop and variety.	Source or C. I. No.	1919–20				1920–21				1921–22		Average number of infected plants.		
		Plants.		Culms.		Plants.		Culms.		Plants.				
		Total.	Infected.	Total.	Infected.	Total.	Infected.	Total.	Infected.	Total.	Infected.	Years averaged.	Plants.	Infected.
WINTER WHEAT.														
		No.	*P. ct.*	*No.*	*P. ct.*	*No.*	*P. ct.*	*No.*	*P. ct.*	*No.*	*P. ct.*		*No.*	*P. ct.*
China	180					50	2.0	293	0.3	9	11.1	2	2	3.4
Flint	6612					60	25.0	605	4.5	17	41.2	2	22	28.6
Fulcaster	6162	35	0	165	0	44	0	237	0	14	0	3	0	0
Fulcaster	Tenn.					52	11.5	269	3.7	15	0	2	6	9.0
Fultz	3598	40	45.0	364	31.3	57	78.9	340	54.4	16	18.8	3	66	58.4
Gipsy	5579					53	3.8	291	1.0	16	0	2	2	2.0
Goens	3498					59	6.8	366	1.1	17	5.9	2	5	4.6
Harvest Queen	5957	48	85.4	434	79.5	116	93.1	1,094	87.1	104	88.5	3	241	89.9
Do	Ill.	41	75.6	420	73.8							1	31	75.6
Honor	6161					52	0	290	0	8	12.5	2	1	1.7
Hussar	4843									46	0	1	0	0
Do	6553									29	0	1	0	0
Illini Chief	5406					58	0	348	0	14	0	2	0	0
Jones Fife	5608					45	86.7	284	75.7	14	42.9	2	45	76.3
Kanred	5146	37	5.4	217	.9	54	0	369	0			2	2	2.1
Martin	1974					61	9.8	350	2.0	12	0	2	6	8.2
Do	4463									60	0	1	0	0
Mealy	5824	45	2.2	196	1.0	53	17.0	273	.7	15	13.3	3	12	12.6
P–1066	5879									6	0	1	0	0
P–1068	5880									15	0	1	0	0
Poole	3489	46	0	189	0	56	0	313	0	14	0	3	0	0
Purplestraw	1915					62	66.1	425	42.6			1	41	66.1
Red May	5596	39	0	229	0	45	0	242	0	14	0	3	0	0
Red Rock	5976	43	0	222	0	50	0	267	0	13	0	3	0	0
Red Wave	5582					48	72.9	313	36.1			1	35	72.9
Treadwell	3527					45	0	419	0	10	0	2	0	0
Turkey	5966					58	0	485	0	17	0	2	0	0
SPRING WHEAT														
Baart	1697	45	35.6	251	17.1	55	29.1	219	9.1			2	32	32.0
Bobs	4990	43	93.0	257	84.0	124	98.4	541	94.5	30	86.7	3	188	95.4
Bunyip	5012	49	2.0	158	.6	59	5.1	177	1.7	16	31.3	3	9	7.3
Cedar	4117	52	0	210	0	60	6.7	221	2.3	14	28.6	3	8	6.3
Chul	2227					62	0	258	0	14	14.3	2	2	2.6
Comeback	4991	48	0	211	0	64	43.8	223	14.8	15	93.3	3	42	33.1
Cowra No. 3	4119	41	92.7	264	89.8	47	87.2	370	81.4	31	0	3	79	66.4
Defiance	Calif.	43	88.4	317	52.7	61	24.6	242	9.5			2	53	51.0
Dicklow	3663					40	50.0	176	30.1			1	20	50.0
Early Defiance	6480	43	0	183	0	55	0	227	0	17	0	3	0	0
Florence	5129	44	0	157	0	46	13.0	187	3.7	19	31.6	3	12	11.0
Galgalos	2398	47	0	264	0	60	0	342	0	16	0	3	0	0
Hard Federation	4733	51	72.5	273	68.1	56	98.2	230	95.2	26	80.8	3	113	85.0
Little Club	4066	50	2.0	235	.9	56	100	324	95.7	45	97.8	3	101	66.9
Marquis	3641	51	7.8	292	2.4	63	3.2	270	1.1	15	0	3	6	4.7
Pacific Bluestem	3019–3	48	4.2	206	1.5	61	4.9	275	1.8	12	33.3	3	9	7.4
Peliss	1584					58	0	169	0	13	0	2	0	0
Propo	1970	51	35.3	247	16.6	64	62.5	322	51.9			2	58	50.4
Sonora	4293	51	2.0	185	.5	57	29.8	272	8.5	16	25.0	3	22	17.7
White Federation	4981	45	88.9	207	84.5	50	100	247	92.7	39	97.4	3	128	93.5
EMMER.														
Black Winter	2337	40	0	108	0							1	0	0
SPELT.														
Alstroum	1773	30	10.0	197	2.0							1	3	10.0

The percentage of infection for the same strains of many varieties was found to be much higher when grown in the greenhouse than when grown

in the field. Tisdale, Dungan, and Leighty (*15*) give the average percentage of infection for the different varieties grown in the field for a number of years. The same strains of some of the varieties grown by them in the field also were grown in the greenhouse by the writer. The average percentages of infected plants grown under both field and greenhouse conditions are given in Table XI.

TABLE XI.—*Percentage of infection of the same strains of wheat, spelt and emmer inoculated with Urocystis tritici and grown in the field at Granite City, Ill., and in the green house at Arlington Experiment Farm, Rosslyn, Va., in the same three years, 1919, 1920, and 1921*

Variety.	Source or C. I. No.	Field.		Greenhouse.	
		Average infection.	Number of years grown.	Average infection.	Number of years grown.
WHEAT.		*Per cent.*		*Per cent.*	
China	180	1.6	2	3.4	2
Dawson	6161	5.8	2	1.7	2
Fulcaster	6162	1.2	3	0	3
Fultz	3598	2.5	2	58.4	3
Gipsy	5579	.7	3	2.9	2
Goens	3428	2.5	2	6.6	2
Harvest Queen	5957	24.9	3	89.9	3
Do	Ill.	18.1	1	75.6	1
Hussar	4843	0	1	0	1
Illini Chief	5406	3.0	2	0	2
Jones Fife	5608	4.8	2	76.3	2
Kanred	5146	2.5	3	2.2	2
Martin	Wn. 1092	0	1	0	1
Mealy	5824	7.3	2	10.6	3
P-1066	5879	.3	2	0	1
P-1068	5880	0	2	0	1
Poole	3489	Trace.	3	0	3
Purplestraw	1915	9.7	2	66.1	1
Red May	5596	1.0	2	0	3
Red Rock	5976	.2	3	0	3
Red Wave	5582	Trace.	1	72.9	1
Treadwell	3527	2.8	3	0	2
SPELT.					
Alstroum	1773	0	2	10.0	1
EMMER.					
Black Winter	2337	0	2	0	1

Many of the varieties which were free from infection when grown in the greenhouse were reported as being slightly susceptible in the field. On the other hand, one of the most susceptible varieties, Harvest Queen (Red Cross), had a three-year average infection of 24.9 per cent in the field. The same strain of Harvest Queen grown in the greenhouse during the same three years had an average infection of 89.9 per cent, or a difference of 65 per cent. Other varieties which showed great differences in percentages of infection were Fultz, Jones Fife, Purplestraw, and Red Wave. The fact that the area quarantined for flag smut is located in the winter-wheat section has precluded any experiments on varietal resistance of spring wheat in the field.

SUMMARY

Flag smut of wheat occurs in the United States, Australia, Japan, China, South Africa, Italy, and Spain. Although heavy losses have been reported from other countries, it has been estimated that in the five years that flag smut has been known in the United States there has been less than 2 per cent of loss in the infested areas.

Flag smut of wheat is caused by *Urocystis tritici* Kcke., which produces sori on the leaves, stalks, and glumes. Badly infected plants do not head. The spores do not germinate readily in tap or distilled water, and special conditions are necessary for uniform germination. Spores kept under laboratory conditions were viable for at least four years. They are able to live through the winter in the soil in the vicinity of St. Louis, Mo. These overwintered spores are capable not only of germinating but of infecting wheat plants.

Sowing inoculated wheat at successive dates in the fall at St. Louis, Mo., resulted in a general decrease in the percentages of infection. There was no infection in the sowing of November 14 or in later sowings.

Infection occurred at 6° to 12° C., 17° to 19°, and 21.5° to 23.5°, but did not occur at 25° to 26.6°. The highest percentage of infection occurred at 21.5° to 23.5°. The most favorable stage of growth for infection was in the seedling stage before the coleoptile was broken and before the seedlings emerged from the soil.

Infection occurred either earlier or in a greater percentage in a few varieties which had been inoculated and cut back at different stages of growth than in the same varieties which had not been cut back.

Fulcaster, Poole, Red May, Red Rock, Early Defiance, and Galgalos remained smut-free during the three years of this experiment; Illini Chief, Treadwell, Turkey, and Peliss, grown for only two years, also were smut-free. The percentages of infection of susceptible varieties grown in the greenhouse were considerably higher than those of the same strains grown in the field.

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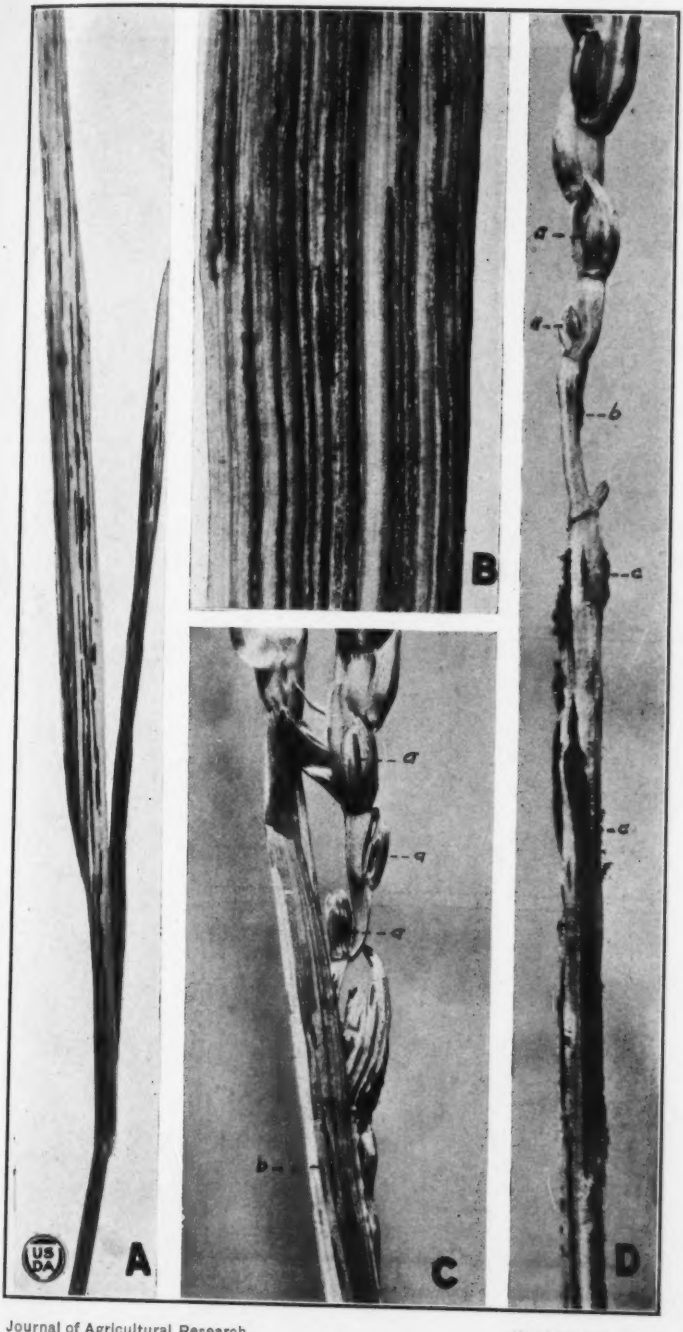
PLATE 1

A.—Upper leaves of a wheat culm infected with *Urocystis tritici*, showing the long stripes, or sori.

B.—An enlarged portion of a wheat leaf infected with *Urocystis tritici*, showing the smut sori.

C.—Flag smut lesions on the lower glumes of an infected wheat head (*a*), and on the leaf sheath (*b*).

D.—Lesions of *Urocystis tritici* on the lower glumes (*a*), the rachis (*b*), and the upper part of the culm (*c*).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C
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a
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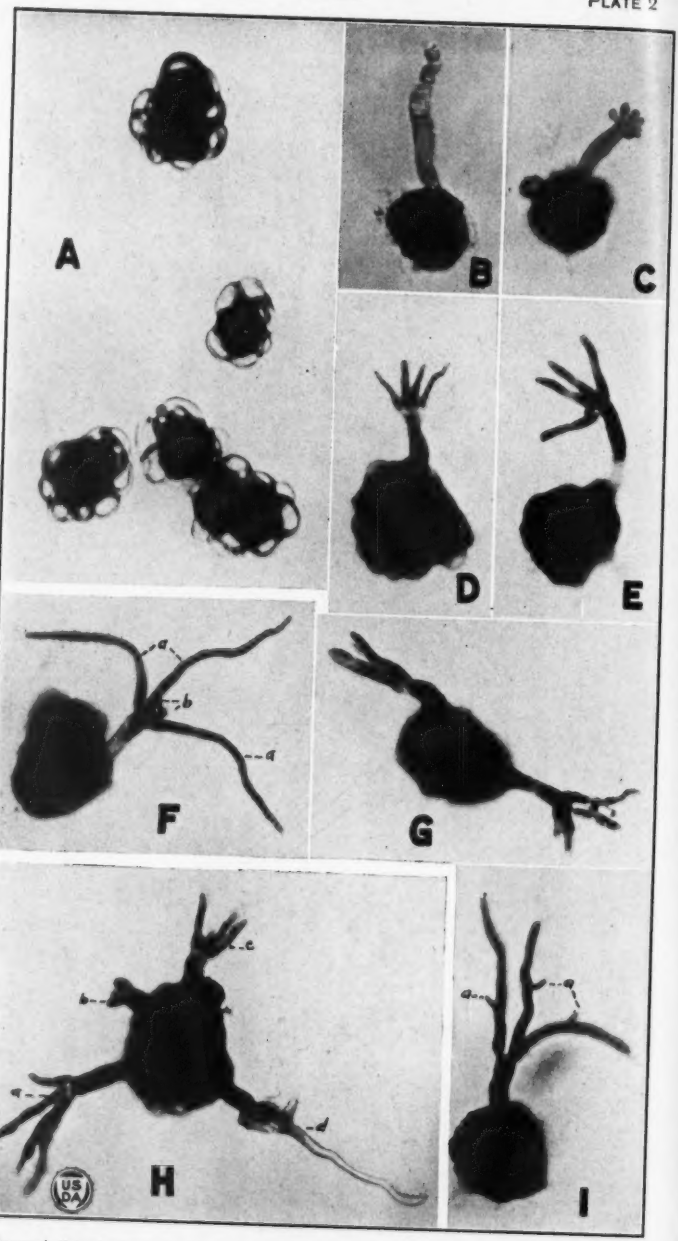
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PLATE 2

Germination of spores of *Urocystis tritici*.

All figures were photographed with 4-mm. objective and No. 10 ocular by Miss Ruth Colvin, Office of Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture.

A.—Spore balls, each with one or two spores, invested by sterile cells.

B.—Germinating spore with promycelium.

C.—Germinating spore with sporidia just forming at apex of promycelium.

D–E.—Germinating spores with sporidia.

F.—Sporidia elongating, forming the so-called infection threads (*a*). Two sporidia (*b*) did not elongate.

G.—Spore ball with two spores germinating, each with sporidia at the apex of the promycelium.

H.—Spore ball with four spores in different stages of germination; (*a*) sporidia beginning to elongate, (*b*) sporidia just forming, (*c*) sporidia formed, and (*d*) sporidium elongated to form an infection thread.

I.—Three sporidia, each with a secondary sporidium.

PLATE 3

A.—A cut-back plant of Comeback wheat showing an infected shoot (*a*) arising from the second node of one of the culms.

B.—An enlargement of a portion of the secondary shoot (*Aa*), showing the typical elongated smut sori.

A
B

STUDIES ON THE PARASITISM OF UROCYSTIS TRITICI KOERN., THE ORGANISM CAUSING FLAG SMUT OF WHEAT[1]

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INTRODUCTION

Flag smut of wheat is a disease which now occurs in a number of the important wheat-growing regions of the world. It has been known to exist in certain countries for many years, in others it has appeared for the first time only recently. In some countries and in some localities, it is not regarded as a very serious disease of wheat, but in others it is a disease of major importance, and its menacing spread is viewed with some alarm.

The disease has been studied by investigators from time to time, but many essential points are lacking in our knowledge of the reaction of the pathogene to its environment and its relationships within the host. The present studies, therefore, were directed toward an elucidation of some of these phases of the parasitism of the causal organism, *Urocystis tritici* Koern.

HISTORY, GEOGRAPHIC DISTRIBUTION, AND ECONOMIC IMPORTANCE

Flag smut is one of the most destructive diseases of wheat. Under certain conditions it may exist in a locality for several seasons before its presence is suspected; and sometimes the disease has only been discovered after it has become epidemic.

McAlpine (*29*)[3] states that flag smut was first recorded from South Australia in 1868, although there is evidence that it was fairly widespread before that time. The disease has now been reported in Australia from Queensland, New South Wales, Victoria, and South Australia. There is as yet no record of its occurrence in Western Australia or Tasmania.

The disease was reported by Hori (*18*) in Japan in 1895, and Sydow and Butler (*47, p. 427*) recorded it from Lyallpur, India, in 1906. Reed and Dungan (*42*) report that it occurs in southern Europe. Dr. E. C. Stakman collected wheat affected with flag smut at Rieti, Italy, June 21, 1922.

[1] Received for publication Nov. 23, 1923. The investigation herein reported was carried on while the writer was a Fuller traveling research scholar of the University of Sydney, Australia. It was made possible by cooperative arrangement with the Bureau of Plant Industry, U. S. Department of Agriculture. The work was done at the University of Minnesota.

[2] The writer takes pleasure in acknowledging his indebtedness to Dr. E. C. Stakman, of the Department of Plant Pathology, University of Minnesota, for advice and helpful criticism during the progress of the investigation. The writer also is indebted to Marion A. Griffiths, of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, who is conducting studies on flag smut, for suggestions and access to her notes in June, 1922.

[3] Reference is made by number (italic) to "Literature cited," pp. 487-489.

Putterill (*40*) found the disease in the Marico district of the Transvaal, South Africa, in 1920. He states that it has probably been present a number of years and that, although the total loss is not great, 50 per cent of the plants may be affected in some fields. Flag smut also is known to occur in China (*49, 50*).

Flag smut was found in the United States for the first time in May, 1919, according to Humphrey and Johnson (*19*). It was found then in a number of fields in the vicinity of Granite City, Madison County, Ill. As a result of a survey made in 1920, the disease was found over an area of approximately 47 square miles (*51*). In 1921 the quarantine zone was extended to include 61 square miles. It is pointed out, however (*45*), that this is not necessarily due to spread of the disease but that a more intensive survey was possible in 1921.

The relative importance of the disease varies considerably in the various countries in which it is now known to exist. It would appear that in Australia the conditions necessary for the perpetuation of the pathogene have been especially favorable, for in that country flag smut is of the greatest importance.

In 1891, Cobb (*9*) stated that the disease was a serious plague. Brittlebank (*4*) in 1920 states that the prevalence of flag smut has increased to an alarming extent in recent years throughout the wheat-growing areas of Victoria, Australia. He states that since stem rust may ruin a crop in a few days under favorable weather conditions, many have considered it as the most destructive disease of cereals in Australia, but flag smut annually takes a toll of from 5 to 70 per cent of the crop, so that the total annual loss from rust sinks into insignificance when compared to that caused by flag smut.

Bartlett (*2*), in a report on a district wheat crop competition in New South Wales, Australia, in 1922, states that flag smut considerably reduced the yield of many crops, and mentions losses as high as 30 per cent of the crop in certain fields.

The amount of damage caused by the disease in any particular locality in a given year often may be closely correlated with environmental conditions and cultural practices. Hence, although it is not possible to give an accurate estimate of the total losses due to flag smut in Australia, there is sufficient evidence to show that the disease is widespread in the States concerned, and fairly conservative estimates place the average annual loss for Australia at 3 per cent.

The situation in India is one of great interest. Butler (*7*) states that the disease is confined to the Punjab. It has been many years since it was first recorded from that country, and, although apparently there have been opportunities for the disease to spread to other localities, it has not been found in any other wheat-growing areas.

Haskell, according to Stakman (*45, p. 162–164*), reports that the disease may cause considerable damage in western Illinois, where as many as 25 per cent of the plants have been diseased in certain fields. However, although the known region of infestation is apparently increasing, the disease evidently is being held in check or even reduced on individual farms.

THE DISEASE

SYMPTOMS

The first symptoms are more or less elongated grayish or dull white stripes on the young leaf. These stripes are slightly raised above the

general surface and are usually first apparent on the lower side of the leaf. They vary from a few millimeters to many centimeters in length (Pl. 1, B) and usually are between the vascular bundles, although occasionally they appear between the vascular bundle and the epidermis. More rarely the peduncle (neck) and inflorescence are attacked (Pl. 1, C). The stripes change from white to dull gray in color, usually from three to four days after the first appearance of the lesions.

The stripes represent individual sori of spores. These sori may remain unbroken for several weeks or less, depending on environmental conditions. The epidermis eventually ruptures and exposes the black mass of spores within. The lesions may appear on plants of practically all ages. McAlpine (*29*) reports the first production of new spores on plants 40 days old. The earliest production of spores observed by the writer occurred on the fifth leaf of a young seedling 29 days after inoculation. Occasionally the parasite gains the ascendancy just as the culms of the host begin to elongate rapidly.

Usually the organism causes considerable stunting of the plant. The leaves become characteristically incurled and twisted, and the plant may be considerably deformed (Pl. 1, D). Even if heads are formed on affected culms, they usually are empty or produce only badly shriveled kernels. Plants which are severely attacked when young may be killed before the unaffected plants are mature; hence their loss easily may be overlooked at harvest time. Frequently not all of the culms are equally severely affected. Many instances have been observed in which normal culms have grown to maturity, although others have been completely destroyed by the parasite. In studies on such plants, under greenhouse conditions, it was observed that these unaffected shoots were those first produced by the plant and that the later-tillering shoots frequently became infected.

THE CAUSAL ORGANISM

Flag smut of wheat is caused by *Urocystis tritici* Koern. It was first designated by Wolff (*57*) in 1873 as *U. occulta* (Wallr.) Rab., and the early records of the disease refer to it under this name. Koernicke (*23, Bd. 16, p. 33–34*), in 1877, first described it as a separate species. The morphological and physiological differences between *U. occulta*, which causes stem smut of rye, and *U. tritici* were again noted by McAlpine (*29*).

Koernicke (*23*), McAlpine (*29*), and others have described the morphological characters of the spores of *Urocystis tritici*. The latter may occur singly or in spore balls, which contain a variable number of spores, although from two to three spores are found most frequently in the ball. The spore balls are dark brown in color and variable in shape, though most frequently globose. They usually vary from 18 to 52μ in length and from 18 to 45μ in breadth. The individual spores are spherical or somewhat oval in shape and vary within the size limits of 12 to 16μ by 9 to 12μ. The spores which occur singly, and the spore balls also, are most frequently completely invested with a layer of sterile peripheral cells, pale brown in color, globose or ellipsoid in shape, and of dimensions 7 to 10μ by 5 to 9μ (Pl. 3, H).

McAlpine (*29*) has described some of the features of the germination of the spores. The writer (*35*) has recently described further phenomena

which may occur during germination. Each spore may produce a promycelium, usually about 30μ in length, which characteristically produces several prolongations which are usually considered sporidia. The sporidia are usually 30 by 5μ in size. A variable number may be produced on the promycelium, although from two to four occur most frequently. These structures may be formed under the surface of the medium in which the spores are germinating, at the surface of the medium, or in the air when only a portion of the spore is in contact with the substrate.

Wolff (*57*) mentions that the germ tubes produced by the sporidia of *Urocystis occulta* may become detached and then may be widely distributed by air. The writer has not observed that the sporidia or the sporidial germ tubes of *U. tritici* ever become detached. The sporidia characteristically germinate while attached to the promycelium. A germ tube is produced from the tip of the sporidium. It is fairly constantly 3 microns in width and under favorable conditions it may grow to a considerable length (from 3 to 4 mm. in solid nutrient media). The sporidia were observed to fuse occasionally, although this did not appear to be a characteristic feature at germination.

PHYSIOLOGICAL STUDIES

INTRODUCTION

There is little need to stress the importance of a knowledge of the conditions which govern germination of the spores of parasitic fungi. Other conditions being favorable, the severity of an outbreak of a disease frequently is determined in the first instance by the number of spores which cause infection of the host. As it has been shown that epidemics of flag smut frequently are traceable to the presence of viable spores in the soil, and as, under those conditions, control by present known methods of seed treatment is not possible, it appears especially desirable that we should have a more complete knowledge of the reaction of the pathogene to its environment, for the factors favoring germination and subsequent growth of the fungus must necessarily be closely related to those primarily responsible for the development of the disease.

The spores of *Urocystis tritici* have long been known to germinate capriciously. The writer (*35*) has referred to the difficulties experienced in obtaining results from spore germination studies and has described a method of stimulating spore germination. Spores which had been presoaked in water for several days germinated readily after the addition of small quantities of certain plant tissues. Tissues from wheat and rye plants were found to be the most effective.

Although not a characteristic feature of the spores of all smut fungi, those of many species do not germinate readily. Potter (*38*) speaks of the difficulty experienced in attempting to germinate spores of *Sorosporium reilianum* (Kuehn.) McAlp. In repeated tests in different seasons and at various times of the year, he noted only slight and irregular germination, and states that rarely did more than 15 per cent of the spores germinate.

Walker and Jones (*52*), in a study on *Urocystis cepulae* Frost, state that "the germination of the fungus spores has been so scanty that the effect of temperature upon the fungus has been necessarily limited to inoculation experiments with infected soil."

Stakman (*43*) records that neither Kühn nor Brefeld was able to secure germination of fresh spores of *Ustilago zeae* (Beck.) Ung., but that Brefeld secured germination in nutrient media, although the spores did not germinate in water until the following spring. Stakman found also that, with the exception of one spore lot, a rest period was required before the spores would germinate. In a review of germination studies on *Tilletia levis* Kühnand *T. tritici* (Bjerk.) Wint., the same investigator reports the uncertain and capricious germination observed by Prévost, DeCandolle, Tulasne, Kühn, Fischer von Waldheim, and Brefeld. Stakman (*43*) reports also that he was rarely able to secure germination of fresh spores, although he was successful in one instance (20 per cent being recorded) and then only when distilled water was used, but that after the spores had passed through a rest period of about eight months' duration almost 100 per cent of them germinated.

It is recognized that distilled water is not always a satisfactory medium for the germination of spores, but this apparently is not always a limiting factor, for it is sometimes as effective as a "nutrient solution," and sometimes even more effective.

MATERIAL AND METHODS

The methods used were essentially the same as those previously reported (*35*). Germination studies were made in Syracuse dishes containing up to 5 cc. of medium.

The spores used in practically all the tests were obtained from material collected in Illinois or from material produced in the greenhouses of the United States Department of Agriculture at Washington, D. C. This material was not more than 12 months old when used in the tests. Spores from material collected in Australia and Italy were used in a number of confirmatory tests made from time to time.

MATURATION

Reference already has been made to the fact that the age of the spore is sometimes a factor in determining its viability. A rest or maturation period apparently is necessary before the spores of many fungi can germinate. The teliospores of *Puccinia graminis* and the oospores of many Phycomycetes are familiar examples.

Davis (*11*), in germination studies on spores of *Ustilago striaeformis* (West.) Niess., states that the spores germinate when properly afterripened. "The spores pass through an after-ripening period varying from 180 to 265 days." The period of germinability is from 53 to 210 days, and "nourishing solutions and decoctions are of no visible value in forcing the germination of these smut spores."

McAlpine (*29*) reports the result of germination studies with *Urocystis tritici* and has suggested that the age of the spore is a possible factor governing germination. He states: "Spores were taken from the wheat plant immediately after maturity and placed directly in water on a slide, but they did not germinate. With material, however, about a month old, and kept seven days on soil, a small proportion of the spores germinated in water after 24 hours." He obtained up to 40 per cent of germinating spores in material several months old.

The nature of this "after-ripening" period is not known, although it generally is assumed to be analogous to the after-ripening period of the seeds of many higher plants. It is possible that with many fungi this period is necessary in order to enable certain changes to take place in the protoplasmic contents of the spore or spore wall.

In experiments designed to hasten the after-ripening process in teliospores of *Puccinia graminis*, Thiel and Weiss (*48*) secured germination of the spores after preliminary treatment with dilute citric acid, several months before germination could be obtained by ordinary methods. They report the work of Crocker, Eckerson, Denny, and others on after-ripening in seeds and permeability of seed coats, in which it has been shown that germination may be delayed until certain definite physiologic changes have taken place in the embryo, or, in other cases, until the seed coats become permeable to water and oxygen.

Thiel and Weiss state that the stimulus to germination of teliospores apparently was not the result of increased permeability of the spore wall, but rather that the citric acid appeared to function as a specific activator of the protoplasm. However, similar treatment of spores of *Urocystis tritici* did not materially increase the amount of germination.

Harrington (*14*) has discussed the effect of artificially drying by heat in hastening the germination of seeds of various cereals which had not after-ripened. Many investigators have mentioned the beneficial effects of such a procedure. A somewhat similar effect after drying in vacuo or over sulphuric acid also has been observed.

While studying the effect of temperature and relative humidity on the viability of the spores of *Urocystis tritici*, the writer obtained some interesting results on the effect of drying fresh spores which had been produced under greenhouse conditions.

Certain infected leaves were closely watched for the change of color in lesions. Two days after the lesions had changed from a whitish gray to a dull, leaden-black color, the infected leaf was removed, cut into strips about 1 cm. in length, and then dried for 48 hours at room temperature (18° to 22° C.) in a dessicator over concentrated sulphuric acid. At the end of this period the spores were shaken out from the leaf section onto the surface of a few cubic centimeters of distilled water, in the manner already referred to, and after three days small quantities of young wheat tissue were added. Eighteen hours later, from 60 to 70 per cent of the spores had germinated. In control dishes, spores obtained from the fresh, undried lesions failed to germinate on the addition of similar amounts of young tissue. Four weeks later, when the infected plants were removed, the sori in the remaining leaves were still unruptured.

It is not known whether there was any effect on the internal composition of the spores or whether the treatment merely acted on the spore envelope, possibly removing certain substances from its surface and thereby facilitating the ingress of water necessary for germination. However, it apparently had the effect of shortening the maturation period, which previously had been considered as essential before the spores would germinate.

It is thus evident that caution should be exercised in stating that certain definite maturation periods are necessary unless the environmental conditions are known. Attention is drawn to this fact elsewhere in this paper in a discussion of the effect of chemical stimulants on the spore.

ACTION OF PLANT ROOTS

It is noted by the writer (35) that the addition of uninjured wheat seedlings to dishes of distilled water containing presoaked spores stimulated germination almost to the same extent as did the addition of small portions of plant tissue.

Several investigators have reported that the presence of the host may profoundly affect germination of spores of parasitic organisms.

Brown (5) has observed that drops of distilled water placed on the surface of leaves and floral structures of a number of plants stimulated the germination of spores of *Botrytis cinerea* Pers. This stimulation was correlated with an exosmosis of electrolytes from the host tissue, as indicated by increased electrical conductivity of the drop.

Chupp (8) states "that the spores of *Plasmodiophora brassicae* Wor. do not germinate at ordinary room temperatures (16° to 21° C.) but do so readily when placed in test tubes on agar with young cabbage seedlings. The presence of the host seems in some manner to exert an influence which to a certain extent takes the place of that offered by a greater amount of heat."

Among some phanerogamic parasites, also, there is evidence that the presence of the host may be necessary in order to supply the stimulus for germination of the seed of the parasite. Duggar (12) has called attention to the work of Koch and Heinricher on germination of seeds of Orobanchaceae. Heinricher makes the interesting observation that the seeds of the parasite germinate in "periods of greatest humidity" when in the presence of the host plant. A substance excreted from the host plant evidently furnished the necessary stimulus, although its nature had not been determined.

More recently McWhorter (32) records the work of Kusano on *Aeginetia indica*, an Orobanchace parasitic on sugar cane. Kusano observed that "the seeds of the parasite lie in the soil for months waiting for the rootlets of some holophytic plant to come near enough to furnish the stimulus necessary for their germination. They can not germinate until the actual contact with a suitable host is attained. The roots of the wrong host will make the seeds start germinating, but they can not complete germination until the roots of a suitable host find them."

In further tests with spores of *Urocystis tritici* it has been found that uninjured seedlings of other plants, namely, field peas and beans, stimulated the germination of spores which had been presoaked in distilled water, although the percentage of germination was not so great as in the instances where wheat or rye seedlings were used (Table I). These spores did not germinate as rapidly as those of the cultures in which cut tissues were used. It is an interesting fact, however, that so many spores germinated, and particularly that nonsusceptible plants should have such a marked influence in this respect. The results are given in Table I. The spores were sown on April 4, incubated at 22° C., and the different seedlings added on April 10.

TABLE I.—*Effect of uninjured plant tissue on the germination of spores of Urocystis tritici presoaked in distilled water*

Number of dish.	Plant.	Percentage of germination on—			
		Apr. 10.	Apr. 11.	Apr. 12.	Apr. 13.
96	Wheat	0	98	.....	.....
97	.....do	0	90	.....	.....
98	.....do	0	85	.....	.....
99	Rye	0	87	.....	.....
100	.....do	0	91	.....	.....
1	.....do	0	72	.....	.....
2	Field pea	0	Trace.	Trace.	.....
3	.....do	0	21	.....	.....
4	.....do	0	2	.....	.....
5	Bean	0	5	.....	.....
6	.....do	0	45	.....	.....
7	.....do	0	11	.....	.....
8	Control of distilled water	0	0	0	0
9	.....do	0	0	0	0

It is possible that such a relationship may have a very fundamental bearing on the question of the epidemiology of the flag-smut disease. The soil solution is of complex and varying composition. Laboratory studies on the germination of spores in soil extract solutions also have shown that the spores will germinate more profusely on the addition of young tissues of various plants. Hence, although it is not possible to duplicate soil conditions in the laboratory, it would appear that at certain times certain facts observed under controlled conditions in the laboratory may at least be duplicated in the field. When there is adequate moisture in the field, the presence of actively growing plant roots alone may be expected to stimulate the germination of many spores of *Urocystis tritici* and thus reduce the amount of viable inoculum between successive crops of the susceptible host. It is a matter of common observation that, under normal conditions, when supplied with an adequate supply of oxygen, plant roots excrete carbon dioxid; when insufficiently supplied with oxygen, the products of anaerobic respiration are carbon dioxid, alcohol, and various organic acids, such as formic, acetic, and lactic.

It is apparent that the most marked stimulation of the spores is to be expected when the environment causes a certain amount of anaerobic respiration of the host roots. It is not known whether the organic acids or the alcohols are primarily responsible for the germination observed, or whether certain other constituents of the plant are effective, for subsequent studies have indicated that small amounts of other volatile substances are effective in this respect also.

EFFECT OF PLANT TISSUE EXTRACTS

Reference has been made to the fact that young tissues of a number of plants stimulated germination of presoaked spores of *Urocystis tritici*.

A number of investigators have observed that plant decoctions form a very suitable medium for the germination of spores of many organisms. Anderson (1) has shown that the germination of pycnospores of *Endothia parasitica* Murr. is slight and uncertain when sown on tap water, rain

water, or distilled water, but that they germinate readily in a decoction of chestnut bark. The action of chestnut bark was not specific, for decoctions of material from trees belonging to other genera, and various nutrient agars, produced the same effect.

Leach (*26*) found that spores of *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara germinated poorly in distilled water, but readily when small portions of the host tissue were present.

Whitehead (*55*) reports that spores of *Urocystis cepulae* Frost germinate slowly when sown in water, but much more rapidly and vigorously when they are sown in onion juice.

In studies on the longevity of spores, Duggar (*12*) has shown that the spores of *Aspergillus flavus* Lk. and *Sterigmatocystis nigra* Britz would not germinate on the surface of distilled water, but when they were transferred to a decoction of beans they germinated readily. Spores of this kind, however, are known to germinate readily on a wide range of nutrient media.

Although plant tissues were so effective in stimulating the germination of spores of *Urocystis tritici*, the optimum effect could not be produced when the tissue was sown with the spores but only after the latter had gone through a "presoak" period. It is suggested that such procedure is necessary because of the nature of the spore wall and also because of the nature of the activating agent. The exact nature of the stimulatory substances in these instances is not known, but from subsequent tests it appears possible that a number of more or less simple substances may be operative. In the earlier tests several sections of wheat tissue were added to a few cubic centimeters of distilled water. Plant tissue decoctions also were effective but these had to be in a suitable dilution.

The concentration of the wheat tissue extracts will vary somewhat according to the conditions under which the seedlings are grown. In the following studies, seed of a number of common wheats was sown between sheets of moist blotting paper at 20° C. Six days later entire seedlings were ground in a meat chopper and the sap expressed with the aid of a hand press. It was found that a concentration of approximately 1 part per 10,000 was the most favorable for germination of the presoaked spores. At higher concentrations germination frequently was delayed. Promycelia sometimes were stunted and distorted and occasionally no spores germinated.

The writer has recorded (*35*) that a steam distillate of a plant infusion also increased spore germination when satisfactory concentrations were used.

Brown (*6*) has recorded the stimulative action of certain volatile products arising from the tissues of a number of plants on the germination of spores of *Botrytis cinerea* Pers., *B. parasitica* (*allii*), *Monilia fructigena* Pers., *Penicillium glaucum* Lk., Fusarium sp. and Collecotrichum. He concludes that "while no exact rule can be laid down in this respect, one can say broadly that some plants give good stimulation, others less so. In some instances volatile materials appeared to inhibit the germination of the spores." He also studied the effects of various chemical substances on the germination of spores of Botrytis and states that "a rough comparison was made of several ethyl esters by testing the effect of a few cubic centimeters of a saturated watery solution of each and of a tenth dilution of the same on the germination of Botrytis in Petri dishes. In all cases inhibition was shown in the presence of the saturated solutions, while stimulation appeared over the tenth saturated solutions of ethyl acetate, malate, and citrate. Any further comparison was not attempted. . . ."

Since it had been shown that the materials which stimulated germination of the spores of *Urocystis tritici* were volatile in steam, tests were made to determine if these materials would volatilize at lower temperatures.

Presoaked spores of *Urocystis tritici* were placed on the surface of a few cubic centimeters of distilled water in Syracuse dishes. The dishes were then placed in a vessel which contained a small quantity of expressed sap of wheat seedlings, and the container was then sealed. After 18 hours at 22° C., over 50 per cent of the spores had germinated in each of the three trials. There was no germination in the controls. It is possible in these cases that the stimulus may have been derived from some of the original constituents of the plant sap, or it may have been derived from some of the products of its decomposition, possibly organic acids, esters, or alcohols.

The spores of *Urocystis tritici* did not germinate readily in solid media, although many different nutrient substrata were used. However, presoaked spores germinated in wheat plant extract agar or in agar containing appropriate concentrations of the distillate from such extracts. Attempts were made to sterilize the surface of the spores by washing them in one-half per cent solution of copper sulphate for periods up to two minutes before transferring them to sterile distilled water for presoaking and subsequent transference to the solid media.

Under these conditions it was not found possible to eliminate entirely all bacterial contamination. However, the spores produced normal sporidia, which in turn produced germ tubes which grew to a length of 4 mm. after four weeks at 7° C. The manner of growth was similar to that previously recorded in liquid media. The protoplasmic contents remained segregated at the tip, while the remainder of the germ tube became septate and apparently was devoid of contents. There appeared to be no increase in the amount of protoplasm in the germ tube.

It is not known whether such germ tubes are capable of producing infection. There is the interesting possibility, however, that the infection capabilities of the organism may be increased by such an extended growth period under soil conditions.

NATURE OF STIMULUS

The identification of the activating constituents of the distillates of wheat-plant decoction is very difficult. The stimulus appeared to be most efficient when the distillates were highly diluted. The distillates obviously are complex in composition and the individual components might be expected to function very differently on spores exposed to their action. Several attempts were made to determine if the activating substances belonged to any general group of chemical substances. "Functional" distillates were used, i. e., distillates in which presoaked spores germinated readily. These were acidified with sulphuric acid and redistilled. The original distillates also were made alkaline with barium hydroxid and then redistilled. Subsequent germination tests with these products of the second distillation showed that in each case they were as potent as the original distillate.

It would thus appear either that a number of the original constituents of the wheat plant decoction were operative or that the stimulus came from some ingredient which was unaffected in either acid or alkaline solution.

It was thought possible that small quantities of volatile oils were the effective constituents of the wheat-plant distillates. A series of rather empirical tests then were designed to test the action of a number of volatile oils on the germination of the spores of *Urocystis tritici*.

The spores were sown on the surface of from 5 to 7 cc. of distilled water at from 18° to 22° C., as in the ordinary germination tests. A minute quantity of each substance was then applied on the point of a needle to each dish of spores. A film formed on the surface of each dish, although slightly different amounts of oil were added in each instance.

The materials which stimulated germination of the spores are shown in Table II. Similar tests also were made with salicylaldehyde, butyric acid, acetone, benzol, oil of thyme, ethyl alcohol, methyl acetate, phenol, benzoic acid, lactic acid, acetic acid, and citric acid. After 24 hours at 22° C., from 80 to 90 per cent of the spores treated with the first three substances had germinated. Less than 1 per cent of the spores treated with the other substances germinated.

Several other essential oils, namely, oil of hemlock (bornyl acetate 30 per cent), oil of lemon (*d*-limonene and citral), eucalyptus (cineol 50 per cent), and eugenol failed to stimulate germination.

No spores germinated in the control dishes. It thus appeared that a large number of substances, in appropriate concentrations, might furnish the necessary stimulus for germination of spores of *Urocystis tritici*.

It is obvious that under the conditions of the experiment (in which the Syracuse dishes are packed in the form of a nest, each dish forming a cover for the one immediately below it), the containers are not air-tight and there might be considerable volatilization of certain of the oils during the period in which the spores might be expected to germinate.

TABLE II.—*The effect of surface films of volatile oils on the germination of spores of Urocystis tritici presoaked on surface of distilled water seven days at from 18° to 22° C. and oil applied on December 6*

Number of dish.	Oil.	Percentage of germination on—		
		Dec. 7.	Dec. 8.	Dec. 9.
26	Balsam peru (cinnamein, 50–65 per cent)	67	.....	.....
28	Oil of tansy (thuyl alcohol, 17 per cent)	29	.....	.....
33	Oil of cassia (cinnamic aldehyde, 75 per cent; trace lead and copper)	15	.....	.....
34	Oil of verbena (citral, 50 per cent)	Trace.	.....	.....
35	Oil of bitter almonds (benzaldehyde, 85 per cent)	91	.....	.....
36	Oil of camphor (safrol, eugenol)	Trace.	.....	.....
37	Oil of bergamot	Trace.	.....	.....
38	Oil of lavender	Trace.	.....	.....
39	Oil of pine needles (30 per cent bornyl acetate)	Trace.	.....	.....
40	Control of distilled water	0	0	0
41	.....do	0	0	0

A study was then made of solutions of certain of the above activating substances to determine the optimum concentrations. Certain of these organic substances are quite soluble in water but are highly volatile (e. g., acetone B. P. 56.48° C., alcohol 64°, ethyl acetate 77°); others again are only slightly soluble, while still others are definitely soluble, even though only in slight amounts and less volatile.

In a number of tests benzaldehyde greatly stimulated germination. Its solubility is slight (0.3 part per 100 parts of water at 20° C.), but its boiling point is comparatively high (179.9°). Thus, it appeared to be suitable for making comparative germination tests under laboratory conditions. A half-saturated solution of benzaldehyde in distilled water was prepared and this was used as a basis for subsequent dilutions. The results of one test are shown in Table III.

At a concentration of 3 parts per 200,000, germination was delayed and subsequent growth of promycelia and germ tubes showed characteristic dwarfing and other abnormalities. This experiment was again repeated in duplicate and it was found that the most favorable concentration was again 3 parts per 2,000,000. It was found that spores subjected to concentrations of 3:20,000 for 18 hours at 20° C. revived when transferred to distilled water and germinated in a normal manner, but those exposed to concentrations of 3:2,000 for the same period apparently had been killed.

Germination at the optimum concentration was quite similar to that induced by the addition of small quantities of plant tissue as in previous experiments. In the latter instances, however, the germ tubes eventually grew further than did those in the benzaldehyde solutions.

TABLE III.—*The effect of a solution of different concentrations of benzaldehyde in distilled water on the germination of spores of Urocystis tritici presoaked at from 18° to 22° C. for six days prior to test, and transferred to solutions on January 8*

Number of dish.	Concentration.	Percentage of germination on—		
		January 8.	January 9.	January 10.
85	3 : 20,000	0	0	0
86	3 : 20,000	0	0	0
87	3 : 200,000	0	Trace.	.....
88	3 : 200,000	0	...do....	.....
89	3 : 2,000,000	0	86	.....
90	3 : 2,000,000	0	80	.....
91	3 : 20,000,000	0	Trace.	.....
92	3 : 20,000,000	0	1	.....
93	3 : 200,000,000	0	0	.....
94	3 : 200,000,000	0	0	0
95	Control in distilled water	0	0	0
96	.....do	0	0	0

A similar test with butyric acid resulted in germination of presoaked spores when sown in concentrations ranging from 1 part in 100,000 distilled water (by volume) to 1 part per million. The optimum concentration for germination of presoaked spores appeared to be 1 part per 500,000.

The results of similar tests with other materials more or less soluble in water were not as striking as those given above. It is possible that they may have been less effective in any case, or that the effective range of concentration was more restricted. In some cases, however, it would appear that because of their great volatility the concentrations are more readily changed during the process of preparation of the dilutions and during the course of the experiment; hence their effect would not be so evident in experiments such as this.

RELATION TO PRESOAKING

Reference already has been made to a method of procedure which involved presoaking of the spores and subsequent addition of a stimulatory agent, which resulted in an optimum germination of the spores of *Urocystis tritici*.

Harrington (*14*) recently has reviewed the results of previous investigations on the presoaking of seeds of certain cereals and its relation to germination and subsequent growth of the plant. He also has studied the effect of presoaking "not after-ripened" seeds of cereals. Within certain limits the procedure had a beneficial effect. Many factor relationships apparently are concerned in the process, but the results indicated that changes in seed-coat permeability were chiefly involved. This author also mentions the work of Eberhardt, who has drawn the interesting conclusion that, although seed-coat permeabilities are increased, protoplasmic alterations also must be involved, as the effect was observed throughout the subsequent growing period.

In a test to determine whether constant association with benzaldehyde in solution would result in germination of spores, results were obtained which were analogous to those previously reported by the writer (*35*) when plant tissues were used.

In a few cases slight germination was noted after six days at 15° C., but in the majority of instances no spores germinated. These spores were viable, for they germinated on the readdition of the activating agent. It would appear that the activating agent had volatilized before it could penetrate and act upon the spore contents. This would explain why spores germinate more readily after presoaking than when sown directly on an activating solution. In the latter instance care has to be taken to prevent loss by volatilization, or allowances have to be made for it by using a more concentrated solution. In such a case there is danger that the more concentrated solutions may be more toxic to the spores.

Thus, it seems most probable that the period of presoaking of spores in water results in an increased permeability of the spore envelope, as the spores respond with apparently equal vigor to the action of a stimulus within a presoak period of from 3 to 10 days at 20° C. This applies equally to spores sown on the surface of water and those totally immersed. There is undoubtedly some action on the spore contents also, but this appears to be only slight until after a sufficiently long period, when the spores germinate without the action of any special stimulus.

ACTION OF STIMULUS

The above results have indicated that many different substances stimulate the germination of the spores of *Urocystis tritici*. They differ widely in chemical composition, for they include acids, alcohols, ketones, aldehydes, and esters. There is no one individual substance which appeared to function as a specific activator of the protoplasm, such as was suggested by Thiel and Weiss (*48*) in their studies on teliospore germination.

It is possible that these substances function partly by increasing the permeability of the spore envelope, although this appears improbable, as the presoak period is not materially shortened by their use. It would appear more probable that they function mainly in increasing the permeability of the protoplasmic spore contents, and thus allow a more

ready ingress of the water necessary for germination of the spore. Without the action of the stimulatory materials, germination in distilled water either is delayed or may not occur at all. The exact nature of the action of these substances on the spores is not known. However, they all have the common property of reducing the surface tension of water.

Larson (*24*) has shown that the surface tension of the medium may have a very definite effect on the cultural habit of certain bacteria. Pellicle-forming bacteria, such as those of the *Bacillus tuberculosis* and *B. subtilis* groups, which habitually grow upon the surface of liquid media, will grow throughout the body of the medium when the surface tension is reduced from 59 dynes (the S. T. of ordinary broth) to from 40 to 45 dynes. The suggestion is made that the bacteria grow throughout the medium when they are properly "wetted."

In a later paper Larson and Montank (*25*) observe that the pathogenicity and viability of the tubercle bacillus also are affected by the reduction of the surface tension of the medium. They suggest that "when the tubercle bacilli are growing diffusely throughout the medium they are wetted and, therefore, when introduced into the animal body, are penetrated by the antibodies or bactericidal substances present and destroyed."

The viability of these organisms is thus dependent on their degree of permeability to bactericidal substances, and this permeability is correlated with a reduced surface tension of the medium.

It has been considered that changes in surface tension of the medium sometimes affect the germination of fungous spores. Many of the stimuli recorded by Duggar (*12*) in his spore germination studies—e. g., alcohol and organic acids—are known to be depressants of the surface tension of water. He states, however, that "attempts to increase the surface tension by means of small quantities of oil in the water gave only negative results."

Melhus and Durrell (*34, p. 132–134*) recorded stimulation of germination of urediniospores of *Puccinia coronata* Cda. by the use of vaseline and paraffin oil. They state that "just how this oil brings about this stimulation is not known, but it is apparent that the surface tension of the water is changed."

Studies by the writer indicated that changes in the surface tension of the medium appeared to have but little effect on the germination of spores of *Urocystis tritici*. Aqueous solutions of sodium ricinoleate and sodium oleate were used. The surface tensions ranged from 72 dynes to 38 dynes, but no significant results were obtained. It was found, however, that very dilute solutions of a number of soaps resulted in germination of the spores (Table IV). Observations were not made on the surface tension of these solutions which were more effective than distilled water but less effective than many of the stimulatory substances discussed above.

Sodium stearate solution, 1 part to 4,000,000, had the most marked effect. It is evident that in such a concentration there is only a very slight change in the surface tension of the fluid as measured against air.

It is not known whether the soap or the hydrolytic products were effective in these cases. The spores failed to germinate in very dilute solutions of potassium hydrate, and hence the soap itself or the fatty-acid radicle appears to have been responsible for the change.

Observations were made on the surface tension of the solutions of benzaldehyde and of butyric acid when in concentrations suitable for the germination of the presoaked spores of *Urocystis tritici*.

TABLE IV.—*The effect of soap solutions on the germination of spores of Urocystis tritici, 11 months old, sown February 15 at from 16° to 22° C.*

Number of dish.	Medium.	Percentage of germination on—						
		Feb. 21.	Feb. 22.	Feb. 23.	Feb. 24.	Feb. 25.	Feb. 26.	Feb. 27.
55	Dist. H_2O	0	0	0	0	0	0	0
50	.....do	0	0	0	0	0	8	.....
58	Ivory soap, 1:40,000	0	0	0	10	.....	.....	.....
64	Ivory soap, 1:400,000	0	0	0	Trace.	.....	.....	.....
70	Ivory soap, 1:4,000,000	0	0	0	Trace.	.....	.....	.....
59	Pot. palmitate, 1:40,000	0	0	0	7	.....	.....	.....
65	Pot. palmitate, 1:400,000	0	0	0	80	.....	.....	.....
71	Pot. palmitate, 1:4,000,000	0	0	0	53	.....	.....	.....
60	Sod. stearate, 1:40,000	0	0	0	0	0	0	Trace.
66	Sod. stearate, 1:400,000	0	0	0	92	.....	.....	.....
72	Sod. stearate, 1:4,000,000	0	86	.....	.....	.....	.....	.....
61	Pot. stearate, 1:40,000	0	0	0	10	.....	.....	.....
67	Pot. stearate, 1:400,000	0	0	0	15	.....	.....	.....
73	Pot. stearate, 1:4,000,000	0	0	0	0	0	0	0
62	Sod. oleate, 1:40,000	0	0	0	Trace.	.....	.....	.....
68	Sod. oleate, 1:400,000	0	0	0	20	.....	.....	.....
74	Sod. oleate, 1:4,000,000	0	0	0	Trace.	.....	.....	.....
63	Sod. palmitate, 1:40,000	0	0	0	0	0	0	Trace.
69	Sod. palmitate, 1:400,000	0	0	0	0	0	0	0
75	Sod. palmitate, 1:4,000,000	0	0	0	0	0	0	0

The observations were made with a Du Noüy surface tension apparatus in which the force necessary to pull a ring from the surface of the liquid was measured as a function of the torsion of a wire. No significant differences were observed between the surface tension of water and that of benzaldehyde in a concentration of 3 parts per 2,000,000 parts of water. The surface tension of butyric acid in water solution (1 part per 100,000) differed from that of water by only 3 to 4 dynes. It would appear that the surface tension of these fluids when measured against air is not a satisfactory criterion for comparison of their effect on the germination of the spores of *Urocystis tritici*.

Stiles (*46*) has extensively reviewed the evidence on the nature of permeability of plant tissues and has reported further experiments on the action of alcohols and certain electrolytes on the permeability of such tissues. Both the cell wall and protoplasmic cell contents are concerned in the process, but the constitution of the protoplasm is of fundamental importance. Protoplasm is considered primarily as a complex colloidal system in which the constituents are continually changing their relationships in reference to one another as a result of the action of various physical and chemical forces to which they are exposed.

In such a system surface action is of paramount importance, and surface phenomena are considered to play an important part in changing the permeability of the cell. In discussing the subject of surface tension, this author calls attention to the fact, already noted by Koltzoff and Vernon, that, although the surface tension of a solution be measured against air, in the experiment the surface tension is acting against the outer layer of protoplasm, and it is not possible to determine the surface tension between two immiscible liquids by knowing only the surface tension of each against air.

Hence, it is possible that entirely new relationships may occur within the protoplasmic cell contents as a result of the action of some substance—e. g., benzaldehyde, certain organic acids, ketones, or alcohols—even though the possibility of this action may not always be indicated by a specific change in the surface tension of the solution.

Price (*39*) has made observations on the protoplasmic condition of certain fungous spores. He used the method of dark ground illumination in studies on dormant and germinating spores of Mucor sp., *Melampsora rostrupii* Wagner, *Triphragmium ulmariae* (Schm.) Link. and *Phragmidium disciflorum* James, and has adduced further evidence on the colloidal nature of the plasmic contents of these bodies. He is of the opinion that the entire spore content is protoplasmic and of the nature of an emulsoid colloid. In the dormant spore the protoplasm is gellike, and at germination the most important change is the transformation from the gel state to a hydrosol.

It would appear, from the fact that so many substances stimulate the germination of spores of *Urocystis tritici*, that their action is such as to change the physical condition of the spore contents, facilitating a change of phase similar to that observed by Price and thus finally increasing the permeability of the spore contents.

Only small quantities of the stimulating materials were necessary, but it is possible, even in the high dilutions observed, that, owing to their physical properties, they readily concentrated at the surfaces and that there may have been a quantitative relationship between the actual amount of the substance in solution and the number of spores which responded to the action of the stimulus. This, however, was not apparent in the dilution tests.

It has been pointed out that the spores of *Urocystis tritici* ordinarily germinate slowly. Without the intervention of a stimulus, the spores may require up to 16 days at 20° C. before they germinate. The first stage of germination is generally considered to be the imbibition of water. The relatively impermeable spore wall permits the ingress of water only very slowly, but when the permeability of both spore wall and spore contents is increased, the spores germinate within a few hours. If the evidence justifies us in considering that the stimulatory substances probably cause a physical change of state in the protoplasmic cell contents and thus increase permeability, we might expect that this action probably is not specific for *U. tritici*. The spores of other fungi which do not germinate readily in water, or which seem to require a rest period before germinating, might also be stimulated by the same substances.

Preliminary tests were made with the spores of *Urocystis occulta*, *Colletotrichum lindemuthianum*, and the teliospores of *Puccinia graminis*. Evidence was obtained that they also respond to such treatment. Such results indicate that caution is necessary in concluding that definite rest or maturation periods are necessary for the spores of such organisms, without taking into consideration the nature of the environmental conditions to which the spores have been subjected.

The methods used in the studies of spore germination in *Urocystis tritici*, which involve the use of surface films or highly diluted solutions of stimulatory substances, would appear to indicate a fruitful mode of attack in studies on the germination of other forms. The practical importance of the method in its relation to pathogenicity studies with certain other fungous spores requires no further comment.

RELATION TO HYDROGEN-ION CONCENTRATION

Many investigators have studied the effect of the acidity of the medium on the germination of fungous spores, but little is known concerning the behavior of the smut fungi in this connection.

Webb (*54, p. 326*) has stated that the spores of *Ustilago avenae* (Pers.) Jens. did not germinate readily in potassium phosphate solutions of different hydrogen-ion concentrations. In mannite solutions, however, the spores germinated within the range P_H 2.4 to 8.2 with an optimum at P_H 6.2. Sporidia were formed most abundantly from P_H 5.4 to 7.0.

The writer has observed that the spores of *Urocystis tritici* did not germinate readily in potassium phosphate solutions, but they did so in certain of them after the addition of small quantities of a stimulatory agent.

Spores were sown in approximately 1½ per cent potassium solutions. Varying amounts of $\frac{M}{5}$ potassium hydrate and $\frac{M}{5}$ phosphoric acid were used in making a series in which the hydrogen-ion concentration varied from P_H 2.4 to 9.6. Final selection of the solutions was made after potentiometer determinations of the hydrogen-ion concentrations.

Two series of observations were made. In the first the spores were sown directly in the solutions and five days later a film of benzaldehyde was added to each dish in the manner already described. In the second series the spores were presoaked in distilled water for five days before they were transferred to the phosphate solutions. Benzaldehyde was then added as in the first instance. The spores were nine months old at the time of testing, and the cultures were kept at from 20° to 22° C. throughout.

It is observed from Table V that the spores germinated within a rather restricted range of hydrogen-ion concentrations. When they were sown directly into the phosphate solutions, they germinated within the range P_H 4.1 to 7.1. Presoaking before transfer appeared to enable the spores to germinate in more acid solutions than in the first series, for a few spores germinated in a solution at P_H 3.6. Within the same ranges, also, more presoaked spores germinated than spores which were sown directly on the phosphate solutions.

TABLE V.—*The effect of hydrogen-ion concentration on the germination of spores of Urocystis tritici sown on phosphate solutions (A) and presoaked in distilled water before transfer to phosphate solutions (B)*

P_H.	Percentage of germination.		P_H.	Percentage of germination.	
	A.	B.		A.	B.
3.2	0	0	5.4	27	78
3.2	0	0	5.7	38	82
3.6	0	[a] Trace.	5.7	45	71
3.6	0	[a] Trace.	6.4	14	12
4.1	6	22	6.4	11	16
4.1	3	34	7.1	[a] Trace.	0
5.1	40	92	7.1	[a] Trace.	0
5.1	32	85	8.3	0	0
5.4	30	68	8.3	0	0

[a] Less than 1 per cent; no sporidia formed.

The optimum concentration for germination appeared to be between P_H 5.1 and 5.7. In those concentrations in which only a few spores germinated—namely, P_H 3.6 and P_H 7.1—no sporidia were produced.

These results correspond fairly closely to those which had been obtained previously when small quantities of young wheat-plant tissue were added to the solutions, although in these studies the procedure enabled the spores to germinate in solutions which were originally slightly more alkaline than those in which germination occurred in the present experiment.

The previous treatment of the spores is thus an important factor in determining their subsequent behavior at germination. Hence, it might be expected that previous exposure to other environmental conditions might also have an effect on the nature and extent of germination in solutions of different hydrogen-ion concentration.

The above results, however, indicate a set of conditions within which the spores of *Urocystis tritici* might be expected to germinate readily. There apparently is no information as to the behavior of the spores in relation to various conditions of soil acidity. Laboratory experiments thus merely indicate that a somewhat similar relationship might be expected to occur in the field. Other conditions being favorable, the spores germinate most readily when in contact with slightly acid solutions.

VIABILITY OF SPORES

It is not definitely known how long the spores of *Urocystis tritici* may retain their viability in the soil, although there is evidence that some spores may live for a considerable number of years. McDiarmid (*30*) reports a case of infection in a crop after an interval of seven years. The seed was treated with formaldehyde solution and was sown on land which seven years previously had borne a diseased crop, but which had not been cropped since that time. Such evidence, of course, is far from conclusive, since there is a possibility that spores may have been blown on to the land in the meantime. McAlpine (*29*) reports some pot experiments in which he was unable to secure infection with spores that were more than 12 months old, although he does not consider these results as entirely conclusive.

In experiments made by the writer it appeared that very few spores were viable after they had been kept 28 months in the laboratory. Repeated tests were made with spores which had been kept in the laboratory for periods ranging from three to nine years, but in no instance did any of the spores germinate. On the other hand, there is a general impression that the spores will retain their viability for several years. It thus appears that environmental conditions may have a very marked influence on the viability of the spores.

Several investigators have studied the effect of controlled temperature and moisture conditions on the viability of the spores of a number of plant pathogenes. Recently Peltier (*37*) has observed that the viability of the urediniospores of *Puccinia graminis tritici* Form III is profoundly affected by the relative humidity and the temperature to which the spores are exposed. He found that the spores retained their viability longest within a range of medium relative humidities.

In experiments on the viability of the spores of *Urocystis tritici*, the writer has used spores of different ages and from different sources, under controlled conditions of temperature and relative humidity. Relative

humidities of 0, 10, 25, 50, 75, 90, and 100 per cent were secured by the use of sulphuric acid in a series of dilutions, according to the method described by Wilson (56). The solutions were placed in wide-mouthed bottles. Infected leaves were cut into sections about 5 mm. in length, and were then suspended over the solutions in small paraffined containers fastened to the undersurface of the corks, the latter being sealed down with paraffin. The bottles were placed in incubators at the following temperatures: 5° to 7° C., 18° to 21°, 26.5° and 37°. The results of early experiments are not recorded, as the spores did not germinate readily in any known nutrient solution. The spore material used in the following experiment had been kept for eight months in the laboratory prior to the commencement of the experiment. Germination tests were made at approximately monthly intervals over a period of five months and, on each occasion, small portions of wheat-seedling tissue were used to stimulate the germination of the spores in water by the method already described.

Under the conditions of the experiment there was no marked difference in the amount of germination at each relative humidity within a temperature range of 5° to 26.5° C. At 90 and 100 per cent relative humidities, however, it became difficult to separate the spores from the host tissue. Consequently, there was a greater amount of contamination in these series and the results are not entirely comparable with those obtained in the lower relative humidities. It was observed also that, even after the tissue had been exposed to 100 per cent relative humidity for five months at the temperatures ranging from 5° to 22°, sometimes almost all the spores of certain small batches germinated.

The consolidated figures for the percentage of germination at all relative humidities for each of the three lower temperature ranges (5° to 26.5° C.) indicate a definite relationship between relative humidity and the viability of the spores (Table VI). Seventy-five per cent relative humidity appeared to be the optimum for the retention of viability at these temperatures. The figures obtained for the 50 per cent relative-humidity series indicate that this also is very favorable for the retention of viability. At both higher and lower humidities, comparatively few spores germinated toward the end of the experiment. This was especially true in all cases at the lower humidities under the conditions of the experiment.

TABLE VI.—*The effect of humidity on the viability of spores of Urocystis tritici eight months old at commencement of experiment on November 22*

Approximate relative humidity.	Relative germination on—[a]						Totals for each relative humidity.
	Dec. 13.	Dec. 29.	Jan. 31.	Feb. 27.	Mar. 25.	Apr. 25.	
0	86	85	16	4	3	8	202
10	68	152	22	11	7	33	293
25	6	131	75	105	22	55	394
50	192	177	196	236	149	148	1,098
75	261	208	207	243	202	185	1,306
90	157	25	51	6	63	68	370
100	114	158	43	5	31	6	357
Totals for all temperatures and all humidities	884	936	610	610	477	503	.....

[a] Totals of percentage of germination at each relative humidity for three temperature series (5° to 26.5° C.).

A consideration of the totals for all temperatures and all humidities shows, on the whole, a tendency toward progressive decrease in the relative number of spores which germinated, but at 75 per cent relative humidity there was apparently no significant decrease in the amount of germination during the experiment.

In the 37° C. series, a few spores germinated within a range of from 0 to 50 per cent relative humidity after exposure for five months from the commencement of the experiment.

When tissue containing fresh spores was exposed to the relative humidity series, it was found that drying over concentrated sulphuric acid at first accelerated the germination of a large number of spores, but constant exposure to this condition eventually inhibited germination. Spores exposed to the medium relative humidities reached their maximum germination capacity later than did those in the low relative humidities, but this capacity for germination was retained for over six months in a manner similar to that observed in Table VI.

It was observed also that the spores from each of the three lower temperature ranges (5° to 26.5° C., inclusive), which had been kept for several months in the 50 to 75 per cent relative humidity series, frequently began to germinate three days after their transference to distilled water at 22°. Plant tissue subsequently was added to the water containing these spores just as in the other instances in order to obtain comparative readings throughout. However, it appears that exposure of the spores to the above-mentioned relative humidities causes a change in their physiological constitution, which is apparently similar in some respects to that induced by the various stimulatory agents described above.

It is obvious that a number of limitations are to be noted in the experiments just described. It may well be expected that there are differences in the maturity of spores in the various leaf sections, and possibly also inherent individual differences in spores which are considered equally mature, for spores produced under varying environmental conditions may respond differently to the influence of the controlled environmental conditions of such an experiment. The question arises, also, as to whether the undisturbed spores of the sorus are completely affected by the relative humidity to which the tissue is subjected. Exposed spores undoubtedly would be more readily subject to this influence, although under natural conditions they often may remain in the tissue for considerable periods. In making the germination tests, it evidently is not practicable to maintain absolutely uniform conditions throughout. However, it is believed that a consideration of the consolidated totals of percentage germination at each relative humidity indicates that, although the extreme limits have not yet been determined, medium relative humidities favor the longevity of the spores.

INOCULATION AND INFECTION

SEEDLING INFECTION

McAlpine (*29*) first reported inoculation experiments with this fungus. Infection occurred when spores were dusted on the seed and also when clean seed was sown in infested soil. There is no available information, however, as to the actual conditions under which infection may occur. Reference frequently has been made to the fact that seedlings are susceptible to certain smut fungi only during a very limited period.

The stage of growth of the host at this period is of great importance. McAlpine (*29*) also has pointed out that environmental conditions which favor a prolongation of this critical period are generally conducive to heavier infections. In discussing resistance to bunt, he mentions that differences in susceptibility of the wheat varieties may sometimes be correlated with differences in rapidity of their seedling growth.

In preliminary experiments with dry spores of *Urocystis tritici*, the writer inoculated wheat seedlings of several varieties of wheat known to be susceptible to flag smut (Federation, Marshall's No. 3, and Canberra) and sowed them in moist soil at from 18° to 23° C., but in no instance was infection noted when the coleoptiles were more than 4 mm. in length at inoculation.

As a result of subsequent investigations, it is highly probable that seedlings in later stages of growth may become infected when inoculated with germinating spores and kept in a suitable environment.

AMOUNT OF INOCULUM

Several investigators have called attention to the relationship between the amount of inoculum employed and the subsequent development of the disease. In this connection, McAlpine (*29*) has recorded the results of inoculation experiments with bunt spores. When inoculum was applied at the rate of 1 bunt ball per 5 kernels, there was from 79 to 81 per cent of infection; when applied at the rate of 1 ball per 100 grains, there was from 56 to 58 per cent of infection.

Heald (*15*) has indicated a quantitative relationship between the spore load in seed wheat and the percentage of stinking smut produced in the crop. Using artificially smutted seed, a load of 36,000 to 150,000 spores of *Tilletia tritici* was necessary to produce maximum infection. He suggests that either multiple infection occurs or that there is a chemical mass effect due to the number of spores.

There are some points of similarity between infection of the wheat seedling by the flag smut organism and infection by the bunt organism. Infection may result from inoculum on the seed or from inoculum present in the soil. The germ tubes or infection hyphae penetrate the tissues of the coleoptile and establish parasitic relationships within the young growing tissues of the host.

In pathogenicity studies with *Urocystis tritici*, infection frequently has been difficult to secure by means of dusting the spores on the grain, even though large amounts of inoculum were used, but when the young coleoptiles were inoculated with several platinum loopsful of germinating spores and the seedlings subsequently incubated under favorable conditions, almost complete infection resulted. Hence, a few spores germinating under suitable environmental conditions may be as effective in producing heavy infection as a much larger number in which the percentage of germination is relatively low.

In flag-smut pathogenicity experiments in the past, dry spores have always been used as a source of inoculum on account of the very capricious germination of spores. In laboratory tests it has been difficult to germinate the spores, but they appeared to germinate somewhat more readily under certain conditions existing in the soil. At other times, however, it appeared as if the spores did not germinate readily even under apparently favorable soil conditions. This fact seems to constitute one of the reasons why the flag-smut organism is such an important pathogene

under Australian conditions. If the spores germinated readily, it would be a simpler matter to free the soil of viable inoculum merely by rotation of crops, and control would be largely a question of seed disinfection. At present, however, under Australian conditions, the inoculum in the soil appears to be the major source of infection.

RELATION TO MOISTURE

It is generally conceded in cases of soil infestation by cereal smut organisms, other factors being favorable, that there is a fairly close correlation between the amount of smut in the crop and the amount of moisture present in the soil during the period prior to sowing.

McAlpine (*29*), in flag-smut infection experiments, reports that sowings made in dry, infested soil resulted in 14 per cent of infection when germination of seed of wheat and fungous spores occurred simultaneously, immediately following the first rains. When sowings were made on adjacent plots one month after rain, only 1 per cent of the plants became infected.

Heald and Woolman (*16*), in discussing the relation of moisture to infection of wheat by bunt, state that sowing in dry soil and waiting for rain is better than sowing in a very wet soil. Mackie (*31*) also observed that there may be sufficient moisture in a soil for germination of wheat seed and yet be insufficient for germination of the bunt spores. Hungerford (*20*), in studies on the same disease, reports that there is a very definite relationship between the amount of moisture at seeding time and the amount of bunt in the resulting crop of wheat. A high soil moisture content at planting was conducive to heavy infection. These authors agree that very little infection results if sowing is delayed for some weeks after the incidence of heavy rains. The spores of *Tilletia tritici* rapidly lose their power to infect, particularly if the soil is cultivated frequently.

Jones (*22*) records that the spores of *Ustilago avenae* (Pers.) Jens. do not germinate readily in very moist soils. She states, "Germination percentages were greatly reduced at 80 per cent of the water-holding capacity."

Walker and Jones (*52*) have shown that soil moisture is not a limiting factor in the development of the onion-smut fungus, *Urocystis cepulae* Frost. They state that "a high percentage of infected plants resulted over the entire range in which good germination and growth of the host occurred."

In greenhouse experiments with spores of *Urocystis tritici*, the writer has observed that wheat plants may become infected when seed is sown in infested soil which had been watered constantly for five weeks. The writer has shown in a previous paper (*35*) that the moist spores rapidly lose their viability when subjected to a temperature of 27.5° C. for a few hours. Spores which had been presoaked in distilled water for five days at 20° failed to germinate after exposure for 36 hours at 27.5°. There are thus two possible reasons for reduction of amount of viable inoculum in moist soil: (1) The spores may germinate and perish in the absence of a suitable host and (2) they may lose their capacity for germination.

It has been mentioned above that the spores of *Urocystis tritici* will produce sporidia either in moist air or when totally submerged in water. It is possible that there are certain limits of soil moisture

content within which germination of the spores may occur most readily. However, the fact that the spores can germinate and produce sporidia when entirely submerged in water would indicate that there is not necessarily any upper limit such as has been indicated for the oat-smut organism described by Jones (*22*). Hence, a special study was not made of this factor under soil conditions.

RELATION TO TEMPERATURE

The writer (*35*) has stated elsewhere that the optimum temperature for germination of the spores of *Urocystis tritici* is between 18 and 24° C. It was observed that spores presoaked in water for six days at 20° germinated most readily at 24°, but that spores exposed to constant temperature only, germinated most readily at 18°.

From field observations and the results of laboratory studies it appeared that soil temperatures might have an important influence on the development of flag smut. The following experiments, therefore, were made in a series of soil-temperature tanks.

The containers were of galvanized iron, about 18 inches deep and 8 inches in diameter. Each held approximately 17 kgm. of clay-loam soil, the moisture content of which had been adjusted to 17.5 per cent on a dry-soil basis. The surface of the soil was about 1 inch below the level of the water in the tank, thus making it possible to maintain a fairly uniform temperature throughout the container.

After the first adjustment for moisture, water was applied through a central tube, which extended to a small crock at the base of the container. The soil was not sterilized, because it was known that it did not contain spores of the flag smut organism. A range of four temperatures was used in each series. By means of suitable adjustment, it was found possible to maintain the series fairly constantly within the following ranges: 14° to 15° C., 19° to 21°, 24° to 26°, and 29° to 31°. Although the air temperature of the above-ground portions of the plant ranged from 15° to 29°, it remained fairly constant at 22°. Thus the soil temperature could have only a direct effect on the process of entrance and infection, although it is possible that there also may be an indirect effect on the parasite due to changes in the physiological condition of the host induced by high soil temperatures.

Canberra, an Australian wheat susceptible to flag smut, was used throughout the test. The seed was treated three minutes in a 1½ per cent solution of copper sulphate and, because of the low percentage of germination, only germinating seeds were planted. From 20 to 24 such seedlings were sown in each container at a depth of from one-half to three-quarters of an inch. Four containers were used at each temperature, a single container being used for each test. The spores used for inoculation were approximately six months old and were known to germinate promptly in laboratory tests.

In the first series (pots 1, 5, 9, and 13), the seedlings were inoculated with spores which had been presoaked on the surface of distilled water for three days at 20° C. Pots 2, 6, 10, and 14 contained seedlings inoculated with dry spores. Spores which had commenced to germinate were used to inoculate the seedlings in pots 3, 7, 11, and 15. In each of these instances the seedlings were inoculated and then sown immediately at the respective temperatures. The final series (pots 4, 8, 12, and 16) contained seedlings inoculated with germinating spores and sown at 15°.

Six days later they were transplanted to the containers in the temperature series. At this time the first leaf of each plant was approximately 3 inches long.

After the seedlings were planted, waxed paper and cotton wool were placed over the surface of the soil to minimize evaporation until the plants had appeared above the soil. Although it was not possible to maintain equivalent and constant moisture conditions throughout the experiment, it is probable that the moisture content of the soil in all of the containers was practically the same until just after infection had occurred. During the course of the experiment, the containers were weighed once a day and water was added to replace the losses from evaporation and transpiration. The moisture content throughout appeared to be adequate for the growth of the host plants.

The seedlings were planted on January 18 and final observations were made on April 3, as by this date all uninfected plants in the three lower-temperature tanks had headed. The plants kept at from 29° to 31° C. were removed from the influence of high temperature on this date, but no smut developed subsequently. The results of the inoculations are given in Table VII, and the condition of the plants when approximately 10 weeks old is shown in Plate 2. In estimating the percentage of smutted plants, all that were smutted or partly smutted are included.

The disease appeared in plants of various ages. The first lesions were observed on two plants in pot 8 and in one in pot 12, 29 days after inoculation (Pl. 1, A). The fifth leaf of both plants was heavily infected. It is observed that all of these plants were inoculated at 15° C. and were subsequently transferred to higher temperatures. Other plants were observed in which the fourth leaf contained typical lesions of the disease. The lesions on these plants were evident 32 days after inoculation.

In contrast to the above, no signs of disease appeared on several plants in pot 1 until 84 days after inoculation.

TABLE VII.—*The effect of soil temperature on the development of Urocystis tritici in seedlings of Canberra wheat*

Pot No.	Temperature.	Inoculum.	Amount infection.[a]	Per cent infection.	Remarks.
	°C.				
1	14–16.....	Presoaked spores.....	12/22	54.5	6 plants partially smutted; 2 plants smutted at heading.
2	.....do.....	Dry spores.....	15/21	71.4	9 plants partially smutted.
3	.....do.....	Germinating spores....	16/18	88.9	3 plants partially smutted.
[b]4	.....do.....	.....do.....	18/20	80.0	2 plants partially smutted.
5	19–21.....	Presoaked spores.....	14/18	66.7	6 plants partially smutted.
6	.....do.....	Dry spores.....	11/21	52.2	4 plants partially smutted.
7	.....do.....	Germinating spores....	16/19	84.2	3 plants partially smutted.
[b]8	.....do.....	.....do.....	17/18	93.8	1 plant partially smutted.

[a] Numerator=number of plants infected: denominator=total number of plants.
[b] Seedlings transferred to temperature series after inoculation and six days' growth at 15° C.

TABLE VII.—*The effect of soil temperature on the development of Urocystis tritici in seedlings of Canberra wheat*—Continued

Pot No.	Temperature.	Inoculum.	Amount infection.[a]	Per cent infection.	Remarks.
	°C.				
9	24–26	Presoaked spores	3/17	17.6	2 plants partially smutted.
10	do	Dry spores	2/21	9.5	
11	do	Germinating spores	5/17	29.4	1 plant partially smutted.
[b]12	do	do	9/16	56.3	
13	29–31	Presoaked spores	0/14	0	
14	do	Dry spores	0/15	0	
15	do	Germinating spores	0/21	0	
[b]16	do	do	1/13	7.7	

[a] Numerator = number of plants infected; denominator = total number of plants.
[b] Seedlings transferred to temperature series after inoculation and six days' growth at 15° C.

Occasionally signs of disease were visible on younger tiller shoots before any lesions appeared on the main shoot. Instances occurred on two plants in the 14° to 16° C. series in pot 1, in which presoaked spores had been used as inoculum.

In considering the effect of temperature on the development of the disease, it would appear from Table VII that the heaviest infection occurred in the series kept at from 14° to 16° C. and 19° to 21°. This is apparent whether the inoculations are made with ungerminated or with germinated spores. On this basis alone, there are no significant differences between the results from either of these series. However, judged on the date of the first appearance of the disease and the degree of infection produced in the plant, the series at 19° to 21° more closely approaches the optimum conditions for infection and subsequent development of the smut (Pl. 2).

The most severe infections recorded in the experiment occurred in pots 7 and 8. In these cases, germinating spores were used as inoculum. Practically all the plants were completely affected and the disease appeared in all such plants approximately six weeks from date of inoculation. Plants inoculated with germinating spores consistently became more heavily infected than those inoculated with ungerminated spores.

In the lower temperature series there was a higher percentage of partially smutted plants and in the majority of instances the first appearance of the disease was considerably later than that recorded for the series at 19° to 21° C.

An effort was made to determine if inoculation at low temperature and subsequent transference of the inoculated plants to higher temperatures would favor the development of the disease. The plants in pots 4, 8, 12, and 16 were inoculated at 15° C. and then transferred to various temperature tanks, as previously described. There apparently are no significant differences between the amount of infection at 14° to 16° and at 19° to 21°, although the highest recorded infection, 93 per cent in pot 8, and the earliest recorded infections, two plants in pot 8 and one plant in pot 12, occurred in the transferred plants.

In the higher temperature series, the transferred plants were much more heavily infected than the plants which had been exposed to constant temperature throughout. In the series at 29° to 31° C., the only

infected plant recorded is one which had been transferred. It is to be remarked that, although the percentage of infection is higher in the plants transferred to high temperatures than in those exposed constantly to high temperatures, the number of infected plants is much lower than in those instances in which transfers were made to the lower temperature series.

A histological study of infected seedlings has shown that a period of six days at 15° C. is longer than is required for entrance of the parasite under the conditions mentioned above; hence, it is highly probable that the germ tubes had entered and became established in the plants before they were transplanted. The subsequent decrease in the percentage of recorded infection is due either to the direct action of temperature or to changed physiologic conditions in the host plant.

Walker and Jones (*52*), in discussing the effect of temperature on the development of onion smut, report that "exposure of onion plants bearing incipient infections to a temperature of from 30° to 33° C. for from 12 to 15 days almost entirely checked further development of the parasite." High air temperatures alone were insufficient to check development of the disease, and it was suggested that the inhibitory effect noted above "may be due in part at least to the influence of the environmental conditions upon the metabolism of the host and not entirely to a direct effect upon the fungus itself."

In the experiments with flag smut, the roots and the small basal portion of the shoot only were exposed to the constant temperature ranges. There is no direct evidence that a change in physiological condition of the host was responsible for checking the amount of infection at the higher temperatures, for it is possible that, as the young tissues of central portions of the plant must have been influenced directly by temperature, the fungus also must have been subject to the same influence. Laboratory studies have shown that constant exposure to temperatures above 24° C. is unfavorable to the development of the fungus, and it would appear that in pots 12 and 16 the plants were more tolerant of the higher temperatures than was the parasite.

Under the conditions of the experiment, there appeared to be no significant differences between presoaked and dry spores when used as inoculum. In these cases the only evidence as to the degree of penetration of the fungus is that derived from a consideration of the degree of infection. It appeared that in the majority of instances in which ungerminated spores were used for inoculation at low temperatures, the organism was less successful in reaching the tissues in the region of the growing point, and, in a number of instances, the main shoots escaped entirely. It appeared that the organism persisted in the lower nodes and thus was readily able to infect the young shoots which arose from them.

Previous experiments have shown that the spores will germinate at 5° C. A test was made to determine if infection might occur at this temperature. Twenty seedlings of Canberra wheat, in which the coleoptiles had just appeared, were kept at 5° for several hours, then heavily inoculated with germinating spores of *Urocystis tritici*. They were then kept in a moist atmosphere at 5° for seven days after which they were removed, thoroughly washed, heavily dusted with copper carbonate, and then planted in sterilized soil. Six weeks later 5 per cent of the plants had produced small culms which bore characteristic lesions of flag smut. None of the plants showed any sign of infection on the main stem. They were allowed to head, but no further sign of the disease appeared.

DISTRIBUTION OF THE PATHOGENE WITHIN THE HOST

The distribution of the pathogene within the host has been studied by investigators of other smuts.

McAlpine (*29*) cites an instance in which a barley plant produced heads affected with loose smut, *Ustilago nuda* (Jens.) Kell. and Sw., early in the season. These heads were cut back and a second series of culms was produced. All of these were infected.

Potter (*38*) also has discussed the phenomenon of infection of the nodal branches of sorghum by *Sorosporium reilianum* (Kuehn) McAlp. He examined the buds on individual culms for presence of the parasite. In some cases the basal portion of the culm escaped, and occasionally the top grew away from the parasite, although, in this instance, it usually remained sterile. The regularities in infection of these buds indicated that they were infected early, rather than that primary infection occurred. Potter states that the extent to which the mycelium develops during the first few weeks while the sorghum plant is growing slowly determines the final extent of infection.

Histological studies of the tissues of plants infected with flag smut indicate that the mycelium is frequently observed at the nodes even when the main shoot is of considerable length (fig. 1, d, and Pl. 3, D, a).

In tests on partially infected plants in the soil-temperature series, all infected culms were removed, and it was observed that the new culms which subsequently developed were most frequently infected. In other instances infected shoots were observed to arise from nodes almost 2 inches from the level of the ground and on main stems otherwise showing no sign of infection.

From the above it would appear that those conditions which favor the development of secondary culms might very readily result in an increase in the amount of damage resulting from flag smut.

It is possible that the relatively crowded condition of the plants in the soil temperature containers was sometimes unfavorable to the production of tillers. Several plants, consisting of a single stem only, produced normal heads and thus did not have as full an opportunity of indicating the presence of the parasite as did those plants which produced secondary shoots.

Hecke (*17*) has described a form of infection which he terms "Triebinfektion." He claims to have produced infection of the young shoots of Melandryum by cutting back 2-year-old plants and dusting the crown with spores of *Ustilago violacea* (Pers.) Fuckel. Subsequent shoots were smutted. A similar experiment was conducted with the perennial rye, *Secale montanum*. The plants were cut back to the crown and dusted with spores of *Urocystis occulta* (Wal.) Rab. The shoots which subsequently developed were affected with smut. Hecke suggests that this mode of infection is not restricted to these organisms but that it occurs with other smut fungi.

An unsuccessful attempt was made by the writer to produce flag smut in wheat by a similar method. Twenty-four plants of Federation wheat were cut back to within one-fourth of an inch of the ground level, the soil was removed from the crown and the upper roots, and the plants were heavily inoculated with spores of *Urocystis tritici*. Soil then was added to cover the plants completely, but the smut did not appear in any of the young shoots which subsequently developed. It is possible that the results were negative because few spores germinated. More

conclusive proof, however, might be obtained by inoculating very young shoots with large numbers of germinating spores. This has not yet been attempted.

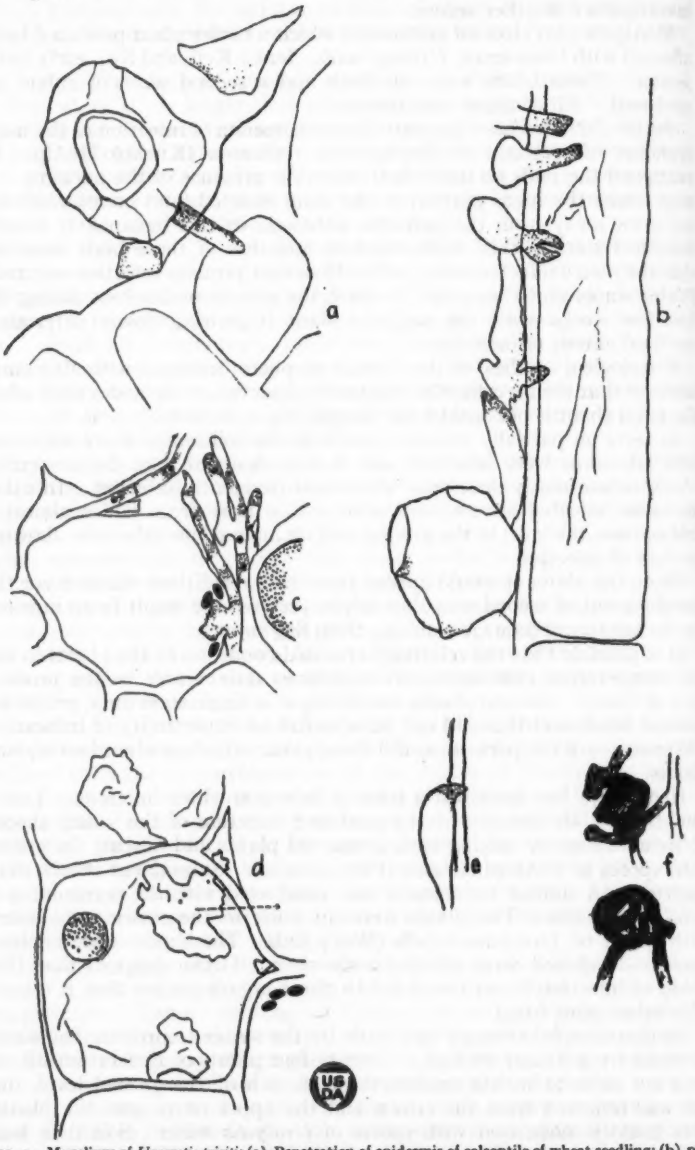

FIG. 1.—Mycelium of *Urocystis tritici:* (a) Penetration of epidermis of coleoptile of wheat seedling; (b) penetration of epidermis of coleoptile of rye seedling; (c) intercellular mycelium in parenchymatous tissues of the leaf; (d) intracellular mycelium (×) in tissues of nodes of infected wheat plant; (e) appressorium-like structure formed by infection hypha on surface of coleoptile; (f) early stage of spore-ball formation. Camera lucida drawings (× 900).

Although the possibility of "Triebinfektion" is admitted, it is felt that such a source of infection of secondary shoots probably is of less frequent occurrence than infection of such shoots by mycelium already present in the plant.

DEVELOPMENT OF THE ORGANISM AND ITS RELATION TO THE HOST

INTRODUCTION

A study of the germination of the spores of *Urocystis tritici* has indicated that there may be considerable variation in the morphological character of the promycelium and of the sporidia. It is an interesting fact that sporidia are formed not only in air but also within a liquid substrate. This rather suggests that the physiological constitution of these bodies is different from those produced by most members of the Tilletiaceae, for it usually is considered that the members of this family produce sporidia only in air.

Brefeld (3, *p. 175, 212, pl. 11*) has discussed the morphological features of the germination of *Urocystis ranunculi*, *U. occulta* (Wallr.) Rab., and *U. filipendulae* Tul. Sporidial structures are produced by these species, but he has questioned whether they should be considered as sporidia and has suggested that perhaps they are sterigmata, which no longer produce sporidia. McAlpine (*29*), on the other hand, states that the structures produced by the promycelium of *U. occulta* should be considered true sporidia because they germinated by putting forth a germ tube. More recently Paravicini (*36*) has stated views similar to those advanced by Brefeld.

The spores of *Urocystis tritici* resemble those of *U. occulta* in many respects. The character of the germination of the spores also is practically the same in each species. Hence, it was thought that a more detailed study of the germinating spores of *U. tritici*, especially with reference to the nuclear phenomena involved, might yield more exact information on the nature of the structures produced and their significance in the propagation of the organism.

A number of investigators have studied the nuclear phenomena involved in various phases of the life history of many smuts. In this connection attention was first directed to the nuclear condition in the young spore. Dangeard (*10*) studied the development of spores in a number of genera of the Ustilaginaceae and Tilletiaceae and found that the young spores consistently contained two nuclei which later fused. This fact has since been confirmed by Maire (*33*), Lutman (*28*), Rawitscher (*41*), and others.

In a discussion of some features of the germination of spores of certain of the Tilletiaceae, Dangeard (*10*) also stated that the nonseptate promycelium of *Urocystis violae* (Sow.) F. de W. and *Tilletia caries* Tul. contained eight nuclei derived from the single fusion nucleus, and a single nucleus then passed into each of the eight sporidia.

A similar observation has been made by Paravicini (*36*) for *Tilletia tritici* (Bjerk.) Wint., *Entyloma calendulae* (Oud.) de B., *Urocystis anemones* (Pers.) Wint., and *U. violae* (Sow.) F. de W. In these forms he states that typically a nucleus wanders to the end of the promycelium and divides there several times, a single nucleus then passing to each of the sporidia.

Lutman (*28*) studied the nuclear condition of the mycelium and nuclear phenomena at the time of spore formation in a number of smut fungi belonging to the Ustilaginaceae and to the Tilletiaceae. He states that in the Ustilaginaceae the mycelium is characteristically multinucleate, whereas the mycelium of the Tilletiaceae frequently contains binucleate cells. In the latter family, *Entyloma nymphaeae* (Cunn.) Set. had binucleated cells and this condition also was characteristic of the older mycelium of *Urocystis anemones* (Pers.) Wint., *Doassansia alismatis* (Nees) Cornu., and *D. deformans* (Setch.). Paravicini (*36*) studied certain members of this family and observed that the sporidia conjugated in pairs, with or without the intervention of a fusion canal, and that a nucleus wandered from one sporidium to the other and thus established a binucleate condition. In *U. anemones*, fusions were seen only rarely, and in *U. violae*, where he observed binucleate sporidia with a fusion canal between them, he was of the opinion that nuclear migration occurred between sporidia also. He states that in both families of the Ustilaginales the binucleate condition arises through conjugation of the sporidia or hyphal cells and is maintained until the spores are formed.

He states that when the spores of *Urocystis anemones* germinated, sporidia were not formed, but he describes the appearance of easily detached mycelial threads which at first contain a single nucleus. Conjugation of these bodies was not observed, but they became septate, each cell contained a single nucleus, and finally certain of these cells became binucleate as a result of the migration of a nucleus from an adjoining cell. He draws an analogy between these mycelial threads and the promycelium of such forms as *Ustilago tritici* and *U. nuda*, although a phylogenetic relationship is not suggested. Paravicini has stressed the fact that conjugation of sporidia is of common occurrence and considerable significance in the sexuality of the smuts.

Lutman (*28*), however, has stated that "it is probable that the parasitic mycelium rarely or never starts from the conjugated conidia or promycelial cells even though they represent the old method of reproduction."

NUCLEAR PHENOMENA AT SPORE GERMINATION

The nuclear phenomena of the germinating spores of *Urocystis tritici* or of *U. occulta* apparently have not been studied. There are some marked morphologic resemblances between the promycelia and sporidia in these forms and the homologous structures produced by such forms as *U. anemones*, *U. filipendulae*, and others mentioned above, but the nuclear phenomena involved at germination appear to differ from those which have been described for such forms by Paravicini.

The writer has made an extensive study of the general characters of the nuclear phenomena associated with the germination of spores of *Urocystis tritici*. Spores in different stages of germination were fixed in Flemming's weaker solution for periods up to two hours, transferred to several changes of water, and then to slides coated with egg albumen. The transfers were made with a platinum loop, this procedure being more satisfactory than direct fixation of the spores on the slide. When the slides were almost dry, a drop of 80 per cent alcohol was added as in the method described by Harper (*13*), and the slides were passed through the regular series of alcohols and then stained. Several staining methods

were used, but Flemming's triple stain and Heidenhain's iron-haematoxylin used alone or with orange G in clove oil counterstain gave the most satisfactory results.

Owing to the thickness of the epispore and surrounding envelope of sterile cells, the first divisions of the nucleus within the spore were not

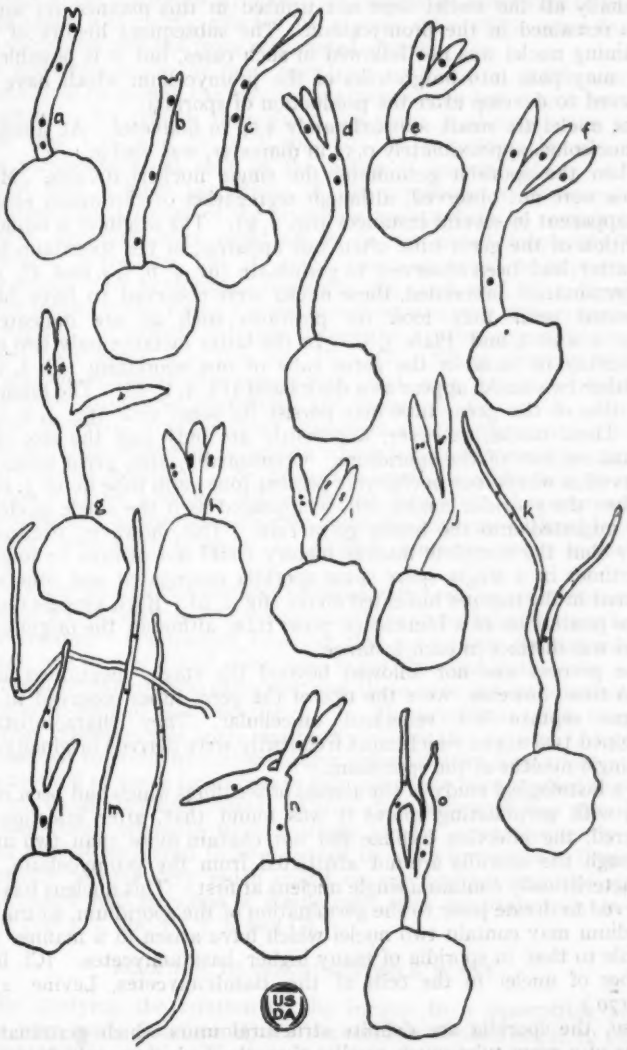

FIG. 2.—Germinating spores of *Urocystis tritici:* (a–f) migration of nuclei to sporidia; (h–j) division of sporidial nucleus; (k–l) binucleate germ tubes; (m) binucleate and 4 nucleate protoplasmic tips of germ tubes; (n–o) conjugation of sporidia. (Camera lucida drawings a–l, n–o, × 700; m×1,000.)

observed. The young promycelium, however, contained a variable number of nuclei, derived from the single nucleus of the spore (fig. 2,

a, b, c, and d). It was not apparent that a single nucleus migrated to the tip of the promycelium and divided there, but division appeared to occur in the spore and in the elongating promycelium.

As the sporidia developed, a single nucleus usually passed from the promycelium into each sporidium (fig. 2, e and f, and Pl. 3, A). Occasionally all the nuclei were not utilized in this manner, for some of them remained in the promycelium. The subsequent history of these remaining nuclei was not followed in such cases, but it is possible that they may pass into outgrowths of the promycelium which have been observed to develop after the production of sporidia.

The nuclei are small, approximately 1.6μ in diameter. At times only the nucleolus, approximately 0.5μ in diameter, was visible.

When the sporidia germinate, the single nucleus divides. Mitotic figures were not observed, although segregation of chromatin elements was apparent in several instances (fig. 2, g). The origin of a binucleate condition of the germ tube often was apparent in the sporidium before the latter had been observed to germinate (fig. 2, h, i, j, and Pl. 3, B). As germination proceeded, these nuclei were observed to have further separated until they took up positions such as are indicated in fig. 2, k and l, and Plate 3, C. In the latter instance only two nuclei are visibly in focus in the germ tube of one sporidium (Pl. 3, C, a); the other two nuclei appear as a dark band (Pl. 3, C, b). The binucleate condition of the germ tube may persist for some time (fig. 2, k, l, and m). These nuclei, however, apparently are only half the size of the original nucleus of the sporidium. Occasionally, also, germ tubes were observed in which four nuclei were present (one germ tube in fig. 2, m).

When the sporidia conjugated, it appeared as if the single nucleus of each migrated into the fusion germ tube. This, however, occurred so rarely that the complete nuclear history could not always be followed. Sometimes in a single spore some sporidia conjugated and others germinated in the manner indicated above (fig. 2, o). Each process resulted in the production of a binucleate germ tube, although the origin of the nuclei was distinct in each instance.

The process was not followed beyond the stages mentioned above. At no time, however, were the tips of the germ tubes observed to have become septate but remained unicellular. They characteristically contained two nuclei which most frequently were derived originally from the single nucleus of the sporidium.

In a histological study of the tissues of seedlings which had been inoculated with germinating spores it was found that, after infection had occurred, the infection hyphae did not contain more than two nuclei. Although the sporidia are not abstricted from the promycelium, they characteristically contain a single nucleus at first. This nucleus has been observed to divide prior to the germination of the sporidium, so that the sporidium may contain two nuclei which have arisen in a manner comparable to that in sporidia of many higher basidiomycetes. (Cf. list of number of nuclei in the cells of the Basidiomycetes, Levine, 27, *p. 164–170.*)

Also, the sporidia are definite structural units which germinate by means of a germ tube much smaller than the body from which it arises. Hence, it seems that they should still be considered as sporidia, although Paravicini (*36*) and others have suggested that homologous structures in other species of Urocystis should not be so considered. In some respects,

however, they differ from the sporidia produced by such genera as Tilletia and they present slight resemblances to an undifferentiated promycelium.

MYCELIUM AND SPORE FORMATION

A study of the epidermis stripped from inoculated colepotiles indicated that entrance was chiefly effected by a germ tube which arose from a single sporidium and not from conjugated sporidia.

The mycelium is for the most part from 1.5 to 2 μ in width. In the early stages of its growth the mycelium is intracellular but it soon becomes intercellular. Its growth throughout the plant is also typically intercellular (fig. 1, c, and Pl. 3, E and G). In the older tissues—e. g., in the parenchymatous cells at the nodes of infected plants—it was observed that a large proportion of the mycelium was intracellular. The intracellular portions of the mycelium may perhaps be considered as haustoria, for they were observed to be associated frequently with intercellular mycelium, but in many cases appeared to represent a characteristic form of dormant mycelium (fig. 1, d, and Pl. 3, D, a).

In the tissues of the young leaf the mycelium was very characteristically intercellular. In heavily infected leaves the hyphae sometimes wedge the host cells apart (fig. 1, c, and Pl. 3, G). When growing rapidly, they are characteristically nonseptate, and may become branched; haustorialike structures sometimes are observed (fig. 2, c). It is a question, however, whether these structures always represent haustoria or whether they represent mycelium which has commenced to take up an intracellular position. The mycelium varies considerably at spore formation. Those portions utilized in the formation of spores are at first narrow and nonseptate, whereas those portions not utilized become vacuolate, somewhat irregular in outline, and are most frequently septate.

The nuclear phenomena were not followed with certainty in all instances, because it was difficult to stain satisfactorily both nuclei and septa in the same mycelium. It appeared, however, that the nuclear content was not constant, one to four nuclei sometimes being observed in the cells. Hyphal fusion frequently was observed, and there were indications that nuclear migrations had occurred (fig. 1, c). The mycelium prior to spore formation, however, was fairly constantly binucleate. Fusion of hyphae was observed to occur at various stages prior to spore formation.

The method of spore formation in this genus has been studied in some detail by Wolff (57) and others. Although it appeared that the spore balls sometimes originate from a single hypha, more frequently two distinct hyphae appeared to be involved in spore formation. Branches arise which become very much coiled on one another and the spores and the spore ball envelope of sterile cells arise simultaneously in this manner (fig. 1, f, and Pl. 3, E and G).

RELATION TO NONSUSCEPTIBLE PLANT

While studying the relation of the fungus to a susceptible plant, studies were made concurrently on the relation of the fungus to plants resistant to flag smut. The relations between certain parasites and nonsusceptible plants have been studied by a number of investigators. Various aspects of the question of resistance and immunity have been studied in this connection, especially by investigators of the cereal rusts.

Stakman (*44*) has studied the action of specific forms of stem rust fungi on immune and susceptible varieties of wheat, oats, and several other hosts and has shown that there is characteristically a very definite antagonism between the protoplasmic cell contents of the immune plant and the parasite.

Woolman (*58*) has recently described the cytologic phenomena of infection of wheat seedlings by bunt caused by *Tilletia tritici*. He states that "*Tilletia tritici* enters the epidermis of the coleoptile of both susceptible and resistant wheat plants grown under conditions for maximum infection, but that in highly resistant varieties it develops no further. Inhibiting factors evidently are active in or just beneath the epidermis."

The writer made a histologic examination of young rye plants which had been inoculated with spores of *Urocystis tritici*. Inoculation experiments have shown repeatedly that rye is resistant to infection by *U. tritici*, but there was no information on the relationship involved. The coleoptiles of young rye seedlings were heavily inoculated with germinating spores of *U. tritici* and placed under conditions of high humidity at 17° C. The epidermis was stripped from some of the seedlings at intervals up to four days, and examined for evidence of entrance of the organism. Portions of the coleoptiles also were fixed and sectioned. A typical section is that shown in figure 2, b. It was very evident that entrance had been effected but that, owing to the unfavorable conditions within the host, development of the organism had been checked. The mode of entrance of the germ tubes resembles the mode of entrance in a susceptible host. Figure 1, e, shows an early stage of entrance of the mycelium. The germ tube swells into an appresoriumlike body and penetrates first through a small opening, somewhat similar to that figured by Waterhouse (*53*) for entrance of sporidial germ tubes of *Puccinia graminis* into the barberry leaf, and Leach (*26*) for entrance of germ tubes of *Colletotrichum lindemuthianum* into beans. No further studies were made at this time on the subsequent history of the infection hyphae of *U. tritici* within the tissues of the immune host.

DISCUSSION AND CONCLUSIONS

A knowledge of the reaction of a pathogene to controlled environmental conditions is fundamental to an understanding of its behavior in nature. It is well known that the severity of many outbreaks of plant disease frequently may be correlated with the environmental conditions to which the pathogene was exposed at a particular period in its life history.

Laboratory and greenhouse studies on *Urocystis tritici* have shown that the viability of the spores is profoundly affected by exposure to controlled temperatures and relative humidity. The germination of the spores, infection, and the subsequent development of the organism within the host also are greatly influenced by the conditions of the environment.

In an infested area in which it is still impracticable to replace the wheat varieties which are susceptible to flag smut by others which are resistant, adequate control of the disease may be expected only by a system of cultural practice which reduces the amount of viable inoculum, which also reduces the possibilities for infection, and finally which tends to reduce the possibilities for serious damage in the crop after infection has occurred.

The present study has furnished data which may be used as a basis for further field experimentation. Also the experimental results may be correlated in part with the present known distribution and seriousness of the disease in certain regions, and thus in conjunction with other data (distribution of wheat varieties, etc.), may serve to indicate the possibility of the disease becoming established in regions in which it does not at present occur.

SUMMARY

1. Flag smut of wheat, caused by *Urocystis tritici* Koern., was first found in Australia in 1868. It is now known to occur also in Japan, China, India, South Africa, southern Europe, and the United States.

2. Flag smut is one of the most destructive diseases of wheat in Australia. It is becoming more widely distributed each year and annually destroys approximately 3 per cent of the total potential wheat crop. Losses up to 70 per cent have been observed in individual fields.

3. The disease lesions may first appear on plants in any stage of growth up to heading. The earliest recorded lesions were observed on the fifth leaf of a wheat plant 29 days after inoculation. Other plants were observed in which the fourth leaf was first affected. The smut may cause considerable deformation of the host. All culms of a plant may not be affected. Under greenhouse conditions, the first formed culms of partially infected plants frequently were free from the disease.

4. The "maturation" period of the spores can be considerably shortened. Fresh spores did not germinate, although they did so after having been dried for 48 hours over concentrated sulphuric acid. Germination tests were made in the manner previously described (*36*).

5. Uninjured seedlings of nonsusceptible plants (field peas, beans, and rye) stimulated the germination of spores which had been presoaked in water. Such stimulation might be expected in the field when the environment causes a certain amount of anaerobic respiration of the plant roots.

6. The expressed sap of wheat seedlings at a concentration of 1 part per 10,000 was most favorable for germination of presoaked spores. The germ tubes of *Urocystis tritici* were observed to grow to a length of 4 mm. in solid media.

7. Surface films of benzaldehyde, salicylaldehyde, butyric acid, and acetone greatly stimulated the germination of presoaked spores. Other volatile substances were less effective. Benzaldehyde, 3 parts per 2,000,000 parts of distilled water, and butyric acid, 1 part per 500,000 parts water, were suitable concentrations for such stimulation.

8. Presoaking the spores appeared to result in increased permeability of the spore enevlope and thus enabled more rapid ingress of the volatile stimulant.

9. The stimulatory action recorded above was not correlated with any definite reduction of the surface tension of the medium as measured against air.

10. It is suggested that the action of the stimulatory agents is mainly such as to cause a change in the physical condition of the protoplasmic spore contents, and thus increase the permeability of the latter.

11. Preliminary tests indicate that films of some of the above-mentioned volatile materials also stimulate the germination of the teliospores of *Puccinia graminis tritici* and the spores of several other organisms.

12. According to the conditions of the experiment, the spores germinated within a hydrogen-ion range of P_H 3.6 to 7.1. Optimum germination occurred within the range P_H 5.1 to 5.7.

13. The relative humidity to which the spores are exposed has a marked effect on their viability. Relative humidities of from 50 to 75 per cent were most favorable for retention of viability. Spores kept at these humidities within a temperature range of from 5° to 26.5° C. frequently commenced to germinate shortly after they were placed in distilled water, without the addition of a stimulatory agent.

14. Wheat seedlings in which the coleoptiles were more than 4 mm. long did not become infected when they were inoculated with dry spores. When seedlings were inoculated with large numbers of dry spores, sometimes no infection occurred, whereas inoculations with few germinating spores at suitable temperatures consistently resulted in heavy infections.

15. Soil temperatures ranging from 14° to 21° C. were optimum for infection of wheat seedlings by *Urocystis tritici*. The most severe and earliest-recorded infection occurred on plants kept at 19° to 21°. Some plants became infected at 23° to 25°. No infection occurred at 29° to 31°. Inoculation at 15° and subsequent transfer of the plants to temperatures above 23° resulted in a decrease of the amount of infection. Some plants became infected at 5°.

16. Studies of the nuclear phenomena during germination of the spores indicate that the spore nucleus divides at germination, the nuclei migrate to the promycelium, and usually a single nucleus wanders into each sporidium. When the sporidia germinate, this nucleus divides, the germ tube becomes binucleate, and this condition may be retained for a considerable period. Germ tubes containing four nuclei were rarely observed. Conjugation of sporidia was sometimes observed. A single germ tube may be produced from the conjugated sporidia, and this germ tube at first contains the two nuclei which migrated from the sporidia.

17. The mycelium within the plant is typically intercellular, and haustorialike bodies are sometimes observed. Branching and fusion of the mycelium occur within the plant. Prior to spore formation, binucleated hyphal cells were observed.

18. Although some spores appear to arise from a single hypha, frequently two distinct hyphae appeared to be involved. Sterile cells and spores arose simultaneously from single coiled structures, and differentiation of the cells occurred as development proceeded.

19. Infection hyphae of *Urocystis tritici* were observed to have entered the tissues of rye seedlings which are known to be immune from the disease.

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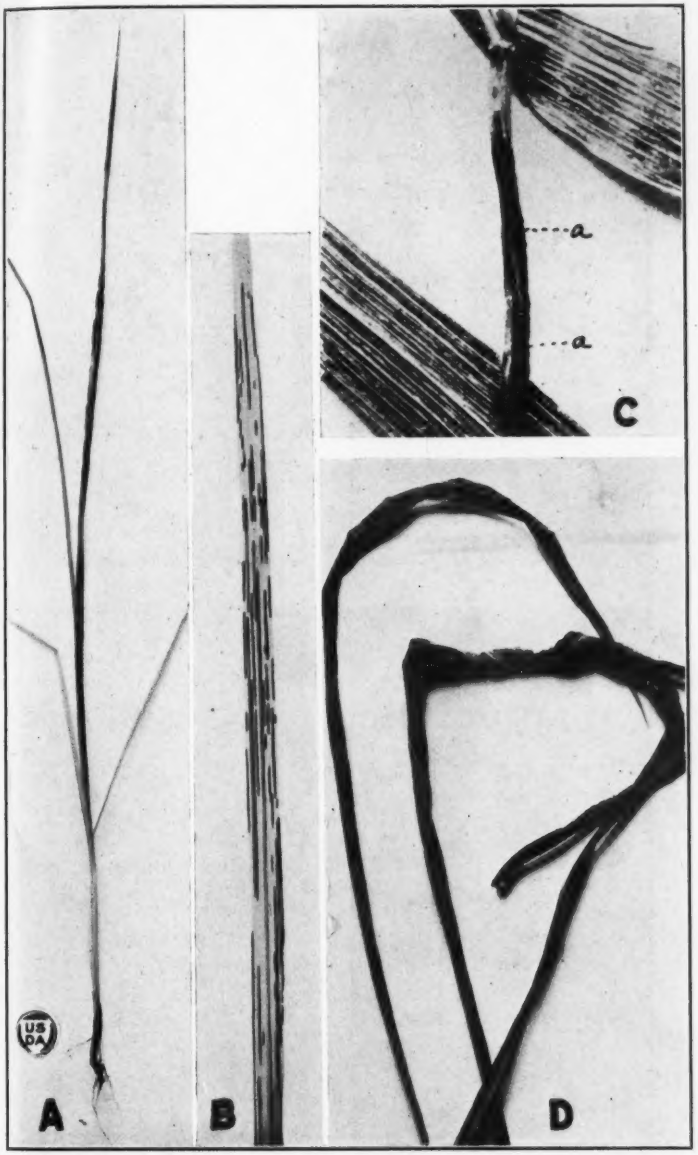
PLATE 1

A.—Young wheat plant affected with flag smut, 29 days from date of inoculation. × 3/5.

B.—Leaf of wheat plant affected with flag smut. (Partly decolorized.) Natural size.

C.—Plant with head affected with flag smut. The darkened areas on the neck (a) represent opened sori filled with spores of *Urocystis tritici*. (Enlarged.)

D.—Portion of plant affected with flag smut showing characteristic curling and twisting of affected leaves. × 3/5.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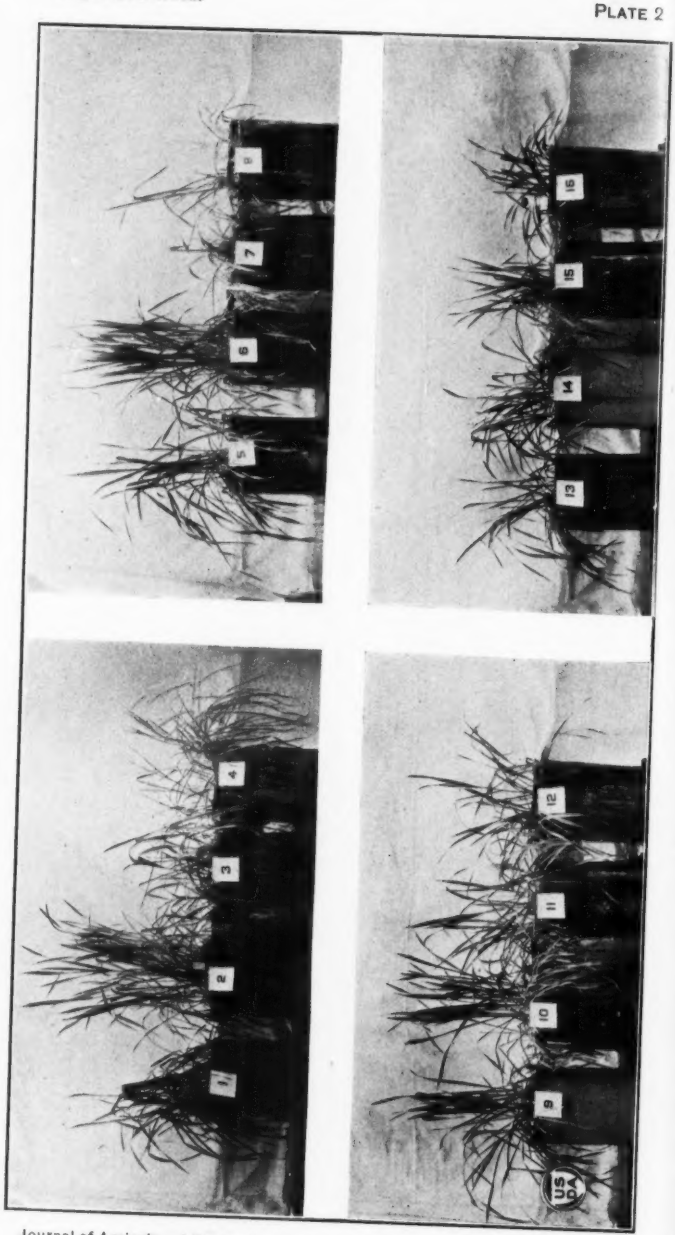
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PLATE 2

The effect of soil temperature on the infection of Canberra wheat by *Urocystis tritici*:

Containers 1–4 at 14°–16° C.
5–8 at 19°–21° C.
9–12 at 24°–26° C.
13–16 at 29°–31° C.

Note destructive action of the pathogene in containers 7 and 8, in which germinating spores were used as inoculum. × $\frac{1}{16}$.

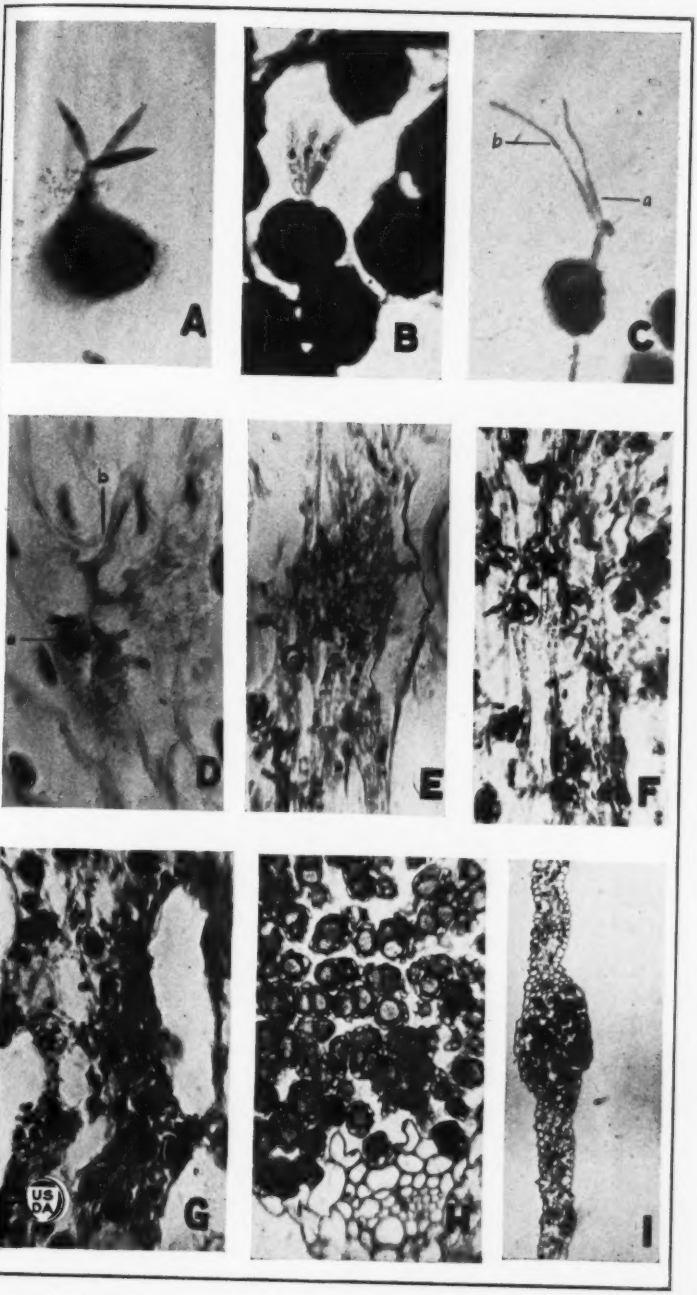
PLATE 3

Germinating spores of *Urocystis tritici*, showing origin of a binucleate condition of the germ tube on germination of sporidium:

A.—Sporidium with single nucleus. × 450.
B.—Sporidium with dividing nucleus. × 450.
C.—Germ tube with 2 nuclei. × 250.

Mycelium and spores of *Urocystis tritici*:

D.—Intracellular (a) and intercellular (b) mycelium at nodes of infected plants. × 450.
E.—Intercellular mycelium, showing first stages of spore formation. × 450.
F.—Spore formation, early stages. × 450.
G.—Spore formation, later stages, formed for most part from intercellular mycelium. × 450.
H.—T. S. wheat leaf showing mature spores and sporeballs in section. × 200.
I.—T. S. wheat leaf showing sorus of spores. × 40.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INHERITANCE OF PETAL SPOT IN PIMA COTTON[1]

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INTRODUCTION

Egyptian cotton, like the related Sea Island cotton (*Gossypium barbadense* L.) is characterized by a conspicuous red spot near the base of the otherwise yellow petal. When the flower first opens, the red pigment appears to be confined to the cells of the epidermis, but soon diffuses into the subjacent tissue. The spot is entirely lacking in most of the varieties of upland cotton (*G. hirsutum* L.).

It has been found that in a hybrid between the Pima variety of Egyptian cotton and the Holdon variety of upland cotton, the parental families having shown, respectively, pronounced development and complete absence of the spot, the spotless condition behaved as a simple recessive.[2] A 3:1 ratio was obtained in F_2 and the behavior of F_3 progenies of individuals representing different points on the F_2 frequency curve confirmed the unifactorial nature of the segregation. The findings of other investigators as to the inheritance of this character are summarized in the publication referred to (p. 31).

In the Pima variety the petal spot is almost invariably well developed, although the character is rather sensitive to environmental influences and there is often variation on the individual plant in the size, shape and intensity of the spot. The normal range was far exceeded, however, by two individuals discovered in 1917 in a field of Pima cotton at Sacaton, Ariz.[3] The inbred descendants of these plants, closely studied during five generations, have shown at most only a very weak development of the spot while in many of the flowers the spot has appeared to be completely absent. Table I gives the mean grade of petal spot for the successive generations of these families and for the normal populations with which they were compared, the means having been based upon the averages of several flowers graded on each plant.

TABLE I.—*Mean grade of petal spot in successive inbred generations of the "spotless" Pima families and in populations of this variety showing a normal development of the spot*

Year.	"Spotless" families.		Normal populations.	
	Number of plants.	Mean grade of petal spot.	Number of plants.	Mean grade of petal spot.
1919	38	1.0±0.04	13	7.5±0.19
1920	140	.9±0.04	.....	.....
1921	29	.9±0.06	34	7.8±0.04
1922	62	.7±0.02	54	7.6±0.03
1923	106	2.0±0.04	111	8.1±0.02

[1] Received for publication Dec. 14, 1923.

[2] KEARNEY, Thomas H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Bul. 1164, p. 21–26, fig. 27, pl. 7. 1923.

[3] KEARNEY, Thomas H. HERITABLE VARIATIONS IN AN APPARENTLY UNIFORM VARIETY OF COTTON. In Jour. Agr. Research, v. 21, p. 239–241, pl. 54. 1921.

Isolation of these Pima families in which the spot is absent or nearly so has afforded opportunity for further study of the inheritance of the character, this time within a single variety. Crosses were made in 1920 between individuals of an inbred Pima family showing normal development of the spot and individuals of the families in which it is very weakly developed. The descendants of these crosses have been studied in the first, second, and third generations and the inheritance of the character in the intra-varietal crosses proves to be similar to that which occurred in the interspecific hybrid Holdon (upland) X Pima (Egyptian).

The heredity of the cotton plant has been studied rather extensively, but definite Mendelian segregation has been demonstrated in relatively few characters. For this reason it is advisable to give in some detail the facts as to the inheritance of petal spot, although, from the standpoint of genetics, the case presents no exceptional features. The subject has, however, a practical bearing, for the possession by an agriculturally valuable strain of Pima cotton of such a character as "spotless" petal would protect the purity of the seed by making it easy to detect the offspring of accidental cross-pollinations with other strains. Proof that this character behaves as a simple Mendelian recessive warrants the expectation that it can be transferred without great difficulty to any desirable strain of Pima cotton.

METHOD OF DETERMINING THE CHARACTER

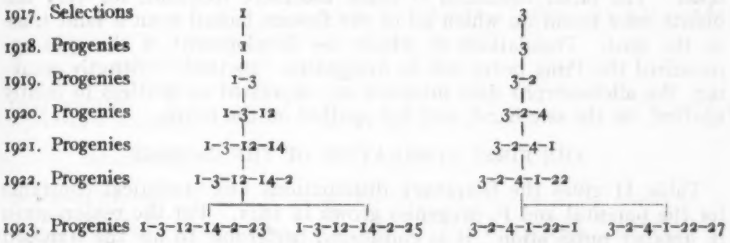
No satisfactory method for the accurate quantitative measurement of the petal spot suggested itself, so a system of grading was employed for the classification of the flowers, as in the earlier study of the upland-Egyptian hybrid. Complete absence of the spot was indicated by grade 0 and its highest development by grade 9. Grades 0, 3, 6, and 9 are represented in Plate 1. The endeavor was to have the grades indicate the total quantity of red pigment present, rather than merely the size of the area or the intensity of the color. Thus a flower having a larger but lighter colored spot might be graded the same as a flower in which the spot was smaller but more deeply colored. It is not assumed that the grading was done with perfect consistency, but it is believed that the deviation from the standard in no case exceeded one full grade and that the results obtained by this method are entirely satisfactory for the purpose of this analysis. For statistical treatment of the data, the method of grading has the advantage of making possible, in a given length of time, the classification of much greater numbers of flowers than could be handled if it were attempted to determine quantitatively the amount of pigment present.

In grading the flowers those representing the two extremes of the scale gave the most trouble. Flowers which appeared to be completely spotless when viewed with the unaided eye showed in some cases, when examined with a hand lens, very faint traces of color in the region of the spot, the red pigment being confined to scattered very small groups of cells. The practice adopted was to grade as 0 all flowers which showed no trace of red when examined in a good light without magnification. Experience indicated that the scale used should have been lengthened somewhat at the upper end, and that if the distinctions had been drawn as finely in this region as elsewhere it would have been necessary to recognize one or possibly two grades above grade 9.

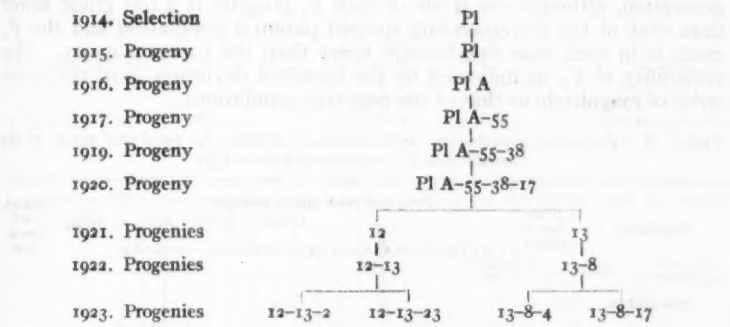
The unit upon which the frequency distributions and statistical constants are based is the average of the grades of several flowers on each individual plant. On a large majority of the plants in each generation 10 flowers were graded, and in no case is the average based upon as few as 2 flowers unless these had differed by not more than one grade.[4]

PARENTAGE OF THE CROSSES AND DESIGNATIONS OF THE POPULATIONS

The two plants selected in 1917 which became the progenitors of the "spotless" families were numbered 1 and 3, respectively. The lines of descent of the successive progenies of these individuals were as follows:



The family which furnished the normal or full-spotted parents of the crosses was descended from plant Pl of 1914, as shown in the following pedigree:


1914. Selection Pl
1915. Progeny Pl
1916. Progeny Pl A
1917. Progeny Pl A-55
1919. Progeny Pl A-55-38
1920. Progeny Pl A-55-38-17
1921. Progenies 12 13
1922. Progenies 12-13 13-8
1923. Progenies 12-13-2 12-13-23 13-8-4 13-8-17

In 1920 plant No. 14 in spotless progeny 1-3-12 was crossed with plant No. 12 in normal progeny Pl A-55-38-17 and plant No. 1 in spotless progeny 3-2-4 was crossed with plant No. 13 in the same normal progeny. The spotless parents were of the second and the normal parents were of the fifth strictly inbred generation. The F_1 progenies, grown in 1921, were designated 1-3-12-14 × 12 and 3-2-4-1 × 13. Two individuals were selfed in each of the F_1 progenies, and gave rise to the following F_2 progenies, which were grown in 1922:

(1-3-12-14 × 12)—20 (3-2-4-1 × 13)—21
(1-3-12-14 × 12)—28 (3-2-4-1 × 13)—24

[4] As a test of the reliability of an average based upon a small number of flowers, comparison was made of the averages for the first 3 and for all 10 of the flowers graded on 100 plants in the F_2 hybrid progenies. The mean departure of the 3-flower average from the 10-flower average was found to be 0.32±0.017 or one-third of a grade. The maximum difference, which occurred in 2 of the 100 plants, was 1.1 grade. It is concluded that averages based upon only 3 flowers would have been sufficiently accurate for most of the purposes of this investigation.

Six individuals were selfed in each of the F_2 progenies and from these 24 F_3 progenies were grown in 1923.

In addition to the hybrid populations, progenies descended from each of the four parents of the crosses were grown each year. Endeavor was made to have the several parental and hybrid populations situated so as to eliminate environmental effect but soil heterogeneity at the Sacaton Station is so pronounced that the endeavor was not wholly successful. It is believed, however, that this factor did not impair the validity of the comparisons.

For convenience, the term "spotless" will be used henceforth to designate populations in which the spot is absent or very weakly developed. The latter condition is much the more frequent, for very few plants were found on which all of the flowers lacked even a faint trace of the spot. Populations in which the development of the spot approached the Pima norm will be designated "spotted." Strictly speaking, the allelomorphs here involved are expressed as spotless to faintly spotted, on the one hand, and full spotted on the other.

THE FIRST GENERATION OF THE CROSSES

Table II gives the frequency distributions and statistical constants for the parental and F_1 progenies grown in 1921. For the reason given in another publication [5], it is considered preferable to use the standard deviation rather than the coefficient of variation as an expression of the variability of a character determined by grading.

The data show almost complete dominance of petal spot in the first generation, although the mode of each F_1 progeny is a full grade lower than that of the corresponding spotted parental population and the F_1 mean is in each case significantly lower than the parental mean. The variability of F_1, as indicated by the standard deviation, is of the same order of magnitude as that of the parental populations.

TABLE II.—*Frequency distributions and statistical constants, for petal spot grade, of the parental and F_1 progenies grown in 1921*

Population.	Number of plants.	Petal spot grade (plant averages).																			Mean.	Standard deviation.
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5			
PARENTAL																						
12 (spotted)	20															1	7	11	1		7.8±.05	0.33
1-3-12-14 (spotless)	12		3	2	5	2															1.2±.11	.58
HYBRID (F_1)																						
1-3-12-14×12	28														2	14	12				7.2±.04	.30
PARENTAL																						
13 (spotted)	14																5	6	3		7.9±.07	.37
3-2-4-1(spotless)	17	1	9	5	2																.7±.06	.39
HYBRID (F_1)																						
3-2-4-1×13	32													1	4	14	11	2			7.1±.05	.44

[5] KEARNEY, Thomas H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID U. S. Dept. Agr. Bul. 1164, p. 13. 1923.

THE SECOND GENERATION OF THE CROSSES

Table III gives the frequency distributions and Table IV the statistical constants of the parental and F_2 hybrid populations grown in 1922. Because of the pronounced segregation in the F_2 progenies, the means and standard deviations were computed separately for the spotted and the spotless class in each progeny.

TABLE III.—*Frequency distributions for petal spot grade of the parental and F_2 progenies grown in 1922*

Population.	Number of plants.	Petal spot grade (plant averages).																	
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
PARENTAL																			
12-13 (spotted)	26															2	14	9	1
1-3-12-14-2 (spotless)	29	1	15	13															
HYBRID (F_2)																			
(1-3-12-14×12) 20	47	4	10	2									3	3	12	4	9		
(1-3-12-14×12) 28	57	1	8	5	1							1	1	3	11	10	11	5	
PARENTAL																			
13-8 (spotted)	28															5	12	10	1
3-2-4-1-22 (spotless)	33	1	20	12															
HYBRID (F_2)																			
(3-2-4-1×13) 21	28	2	2	2	1								1	2	7	4	4	3	
(3-2-4-1×13) 24	47	1	6	3	1							1		4	9	5	7	8	2
4 F_2 progenies as one array	179	8	26	12	3							2	5	12	39	23	31	16	2

TABLE IV.—*Statistical constants for petal spot grade of the parental and F_2 progenies grown in 1922, the constants being given separately for the spotted and the spotless segregates of each hybrid progeny*

Population.	Class.	Number of plants.	Mean.	Standard deviation.
PARENTAL				
12-13	Spotted	26	7.7±.04	0.34
1-3-12-14-2	Spotless	29	.7±.04	.28
HYBRID (F_2)				
(1-3-12-14×12) 20	Spotted	31	6.7±.08	.63
Do	Spotless	16	.4±.05	.30
(1-3-12-14×12) 28	Spotted	42	7.0±.07	.69
Do	Spotless	15	.7±.06	.36
PARENTAL				
13-8	Spotted	28	7.6±.05	.39
3-2-4-1-22	Spotless	33	.7±.03	.27
HYBRID (F_2)				
(3-2-4-1×13) 21	Spotted	21	6.9±.10	.68
Do	Spotless	7	.6±.13	.52
(3-2-4-1×13) 24	Spotted	36	7.1±.09	.82
Do	Spotless	11	.7±.08	.39

Inspection of the frequency distributions of the four F_2 progenies (Table III) shows sharp segregation into a spotless and a spotted group. The percentages of spotless individuals in the F_2 progenies and the departures from the 25 per cent expected with a single factor difference are stated in Table V. It is evident that the departure is significant in none of the progenies and that while the population obtained by combining the four progenies as one array shows a slight excess of spotless individuals, the departure from 25 per cent barely exceeds its probable error.

TABLE V.—*Percentages of spotless individuals and departures from the expectation, in the F_2 progenies grown in 1922*

F_2 progeny.	Number of plants.	Percentage spotless.[a]	Departure from the expectation (25 per cent).
(1–3–12–14×12) 20	47	34.0	9.0±4.66
(1–3–12–14×12) 28	57	26.3	1.3±3.93
(3–2–4–1×13) 21	28	25.0	0±5.51
(3–2–4–1×13) 24	47	23.4	1.6±4.16
4 F_2 progenies as one array	179	27.3	2.3±2.24

[a] The probable error of the percentage is omitted, being the same as that of the departure from the expectation.

Reference to Table IV shows that the mean of the spotless segregates in each F_2 progeny does not differ significantly from the mean of the progeny representing the corresponding spotless grandparent, except in progeny (1–3–12–14×12) 20, in which the spotless segregates gave a significantly lower mean. On the other hand, the means of the spotted segregates in F_2 are in all cases significantly lower than the mean of the progeny representing the corresponding spotted grandparent and approach the means obtained in F_1 (Table II). This, of course, is to be expected if dominance is incomplete, as was indicated by the results in F_1. The incompleteness of the dominance is confirmed by consideration of the variability in F_2. The spotless group in each F_2 progeny is not much more variable than the corresponding spotless parental population, as is made evident by comparison of the frequency distributions (Table III) and of the standard deviations (Table IV). On the other hand, both comparisons show the spotted group in each F_2 progeny to have been much more variable than the corresponding spotted parental population and the spotted groups in F_2 were likewise more variable than F_1 (Table II).

That the heterozygotes of the spotted group are partly distinguishable from the pure dominants is indicated by the marked bimodality of the distributions of the spotted plants in F_2 progenies (1–3–12–14×12) 20 and (3–2–4–1×13) 24 (Table III). Because of the relatively small numbers involved and the nearly complete dominance of the spot, as well as the sensitiveness of this character to environmental influence, it is impossible to resolve the phenotypic ratio of approximately 3:1 into a 1:2:1 ratio. The point will be further discussed in considering the data from F_3.

TABLE VI.—*Frequency distributions for petal spot grade of the parental and F_2 progenies grown in 1923. The grades of the parent individuals are also given and the class of the parent, whether dominant, recessive, or heterozygous, is indicated by the letters D, R, and H*[a]

Population	Number of plants in F_2.	Grade of parent individual.	Class of parent individual.	Petal spot grade (plant averages).																	
				0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9
PARENTAL																						
12–13–23	16	7.9	D																3	11	2	
12–13–2	29	7.3	D																3	15	11	
1–3–12–14–2–23	28	1.2	R			2	10	11	2	2	1											
1–3–12–14–2–25	26	.4	R			1	4	8	7	4	1	1										
HYBRID (F_2)																						
(1–3–12–14×12) 20–14	25	7.7	D														1	4	14	5	1	
(1–3–12–14×12) 20–15	48	7.6	D																12	31	5	
(1–3–12–14×12) 20–19	42	.1	R		15	18	6	2		1												
(1–3–12–14×12) 20–24	37	.1	R	1	5	18	9	4														
(1–3–12–14×12) 20–27	39	5.6	H		2	1	4	1								5	8	5	3	7	3	
(1–3–12–14×12) 20–35	33	5.5	H			3	3		1								9	10	1	5	1	
(1–3–12–14×12) 28–7	34	8.0	D															2	12	16	4	
(1–3–12–14×12) 28–8	46	8.2	D															2	9	27	8	
(1–3–12–14×12) 28–32	48	.1	R			5	13	13	8	8		1										
(1–3–12–14×12) 28–49	47	.3	R		1	15	19	7	5													
(1–3–12–14×12) 28–14	48	5.3	H		6	5	1									5	13	5	3	9	1	
(1–3–12–14×12) 28–53	43	5.9	H			1	4	8	3	1						3	4	11	1	7		
PARENTAL																						
13–8–4	27	8.0	D																2	14	10	1
13–8–17	39	7.2	D																6	21	12	
3–2–4–1–22–1	26	.9	R			1	8	6	6	4	1											
3–2–4–1–22–27	25	.2	R			3	9	9	3	1												
HYBRID (F_2)																						
(3–2–4–1×13) 21–2	42	8.1	D														1		9	21	11	
(3–2–4–1×13) 21–21	46	7.8	D																3	29	14	
(3–2–4–1×13) 21–16	36	0	R			3	8	10	12	3												
(3–2–4–1×13) 21–22	47	.2	R	1	15	21	9	1														
(3–2–4–1×13) 21–7	44	5.6	H		4	4	2									2	12	8	6	6		
(3–2–4–1×13) 21–29	45	6.0	H			1	5	2									13	8	2	9	5	
(3–2–4–1×13) 24–18	43	8.4	D															2	4	23	14	
(3–2–4–1×13) 24–46	41	8.4	D																	12	26	3
(3–2–4–1×13) 24–25	34	.4	R			2	12	11	6		3											
(3–2–4–1×13) 24–48	29	.4	R			4	7	11	3	2	2											
(3–2–4–1×13) 24–33	45	6.1	H			3	4	5	2						1	4	9	9	2	4	2	
(3–2–4–1×13) 24–35	49	6.2	H		3	3	3	5							1	4	12	2	1	6	2	

[a] The table is arranged in two horizontal sections, corresponding to the two original crosses 1–3–12–14×12 and 3–2–4–1×13. Under each cross are grouped together the 6 F_2 progenies descended from each grandparent, the grandparents having been F_1 plants 20 and 28 of the cross 1–3–12–14×12 and F_1 plants 21 and 24 of the cross 3–2–4–1×13.

THE THIRD GENERATION OF THE CROSSES

In each of the four F_2 progenies grown in 1922, flowers on a number of individuals were bagged to insure strict self-fertilization. The selfed individuals were selected for average grade of petal spot as follows: (*a*) Highest, (*b*), most nearly intermediate, (*c*) lowest. Twenty-four F_3 progenies, two representing each condition of petal spot in each F_2 progeny, were grown in 1923. Eight parental progenies were grown in the same plat, each of the original parents of the hybrids having been represented by two progenies.

The frequency distributions for the several parental and F_3 progenies are given in Table VI, the unit having been the average of several flowers graded on each individual plant. The grading was begun on July 14 and ended on August 18.

It is clear from the frequency distributions in Table VI that in every case the behavior of the F_3 progeny accorded with the position of its parent in the F_2 frequency distribution. The eight F_2 parents having average grades higher than 7.5 proved to be pure dominants, the eight F_2 parents having average grades lower than 0.5 proved to be pure recessives, and the eight F_2 parents having average grades between 5 and 6.5 proved to be heterozygous. The percentages of spotless plants and departures from the expected 25 per cent in the segregating F_3 progenies are stated in Table VII.

TABLE VII.—*Percentages of spotless individuals and departures from the expectation in the segregating F_3 progenies grown in 1923*

F_3 progeny.	Number of plants.	Percentage spotless.[a]	Departure from the expectation (25 per cent).
(1-3-12-14×12) 20-27	39	20.5	4.5±4.33
(1-3-12-14×12) 20-35	33	21.2	3.8±4.82
(1-3-12-14×12) 28-14	48	25.0	0±4.21
(1-3-12-14×12) 28-53	43	39.5	14.5±5.03
(3-2-4-1×13) 21-7	44	22.7	2.3±4.25
(3-2-4-1×13) 21-29	45	17.8	7.2±3.85
(3-2-4-1×13) 24-33	45	31.1	6.1±4.65
(3-2-4-1×13) 24-35	42	33.3	8.3±4.90
8 F_3 progenies as one array	339	26.5	1.5±1.61

[a] The probable error of the percentage is omitted, being the same as that of the departure from the expectation.

Reference to Table VII shows that some of the F_3 progenies gave rather wide departures from the expected 25 per cent of spotless individuals. In no case, however, is the departure mathematically significant. If all of the segregating F_3 progenies are considered as one array, the departure from 25 per cent spotless is smaller than its probable error. As was the case in the F_2 progenies taken as one array (Table V), it is the spotless class which is slightly in excess of the expectation.

The spotted portion (grades 5.5 to 9) of the frequency distributions of the heterozygous F_2's (Table VI) is bimodal in 7 of the 8 progenies, indicating that the heterozygotes are partly distinguishable from the dominants. Further evidence that such is the case is afforded by the fact that all of the 8 F_2 plants selected in 1922 as nearest intermediate (grade 5.3 to 6.2) gave F_3 progenies which segregated. While, for reasons stated in discussing the similar condition in F_2, the heterozygotes giving relatively high grades can not be distinguished with certainty from the dominants giving relatively low grades, it may be worth while to note that in the spotted portion of the whole F_3 population there were 163 plants having an average grade not higher than 7 and 86 plants having an average grade higher than 7, a ratio of 1.9:1. While this classification is admittedly arbitrary, it indicates that the phenotypic ratio is 1:2:1.[6]

The statistical constants, as computed from the frequency distributions, of the parental and the homozygous F_3 progenies are stated in Table VIII.

Comparing the means for groups of progenies having a common origin taken as one array (heavy-faced figures in Table VIII), it will be noted that the populations derived from the two dominant parents (12 and 13) of the original hybrids do not differ significantly, and that this is likewise true with respect to the populations representing the two recessive parents (1–3–12–14 and 3–2–4–1). Data given in Table IV show that in the preceding generation also the populations representing, respectively, the dominant and the recessive parents of the hybrids did not differ significantly in their means for petal spot. On the other hand, the population embracing the four dominant F_3 progenies derived from the cross 1–3–12–14 × 12 differs slightly, but significantly, in its means from that of the combined population of dominant F_3's derived from the cross 3–2–4–1 × 13, the difference having been 0.30 ± 0.028. Similarly, the mean for the combined recessive F_3 progenies derived from the cross 1–3–12–14 × 12 shows a small but probably significant difference from that of the combined recessive F_3 progenies derived from the cross 3–2–4–1 × 13, the difference having been 0.21 ± 0.055. In both cases it is the descendants of the cross 3–2–4–1 × 13 which gave the high mean.

There are also significant differences among the several F_3 progenies derived from the same original cross and even between progenies which had had the same F_1 grandparent. Of the two dominant F_3 progenies descended from (1–3–12–14 × 12) F_1 plant No. 20, progeny 20–14 gave a significantly lower mean than progeny 20–15 (difference 0.4 ± 0.067). Of the two dominant F_3 progenies descended from (3–2–4–1 × 13) F_1 plant No. 24, progeny 24–18 gave a significantly lower mean than progeny 24–46 (difference 0.3 ± 0.050). Of the two recessive F_3 progenies descended from (1–3–12–14 × 12) F_1 plant No. 28, progeny 28–32 gave a significantly higher mean than progeny 28–49 (difference 0.5 ± 0.086). Of the two recessive F_3 progenies descended from (3–2–4–1 × 13) F_1 plant No. 21, progeny 21–16 gave a significantly higher mean than progeny 21–22 (difference 1.2 ± 0.072). Thus significant differences are found among the dominant or recessive descendants of each of the four F_1 individuals which gave rise to the F_2's grown in 1922 and the F_3's grown in 1923.

[6] On this basis of classification in the spotted group, the whole segregating F_2 population (8 progenies as one array) comprised 86 dominants, 163 heterozygotes, and 90 recessives.

TABLE VIII.—*Statistical constants for petal spot grade of the parental and the homozygous F_3 progenies grown in 1923*

Population.	Number of plants.	Mean.	Standard deviation.
DOMINANT PARENTAL			
12-13-23	16	8.0 ±.05	0.28
12-13-2	29	8.1 ±.04	.32
Combined	45	8.08±.032	.316
13-8-4	27	8.2 ±.04	.34
13-8-17	39	8.1 ±04	.33
Combined	66	8.12±.028	.338
DOMINANT F_2			
(1-3-12-14×12) 20-14	25	7.5 ±.06	.41
(1-3-12-14×12) 20-15	48	7.9 ±.03	.29
(1-3-12-14×12) 28-7	34	7.8 ±.04	.38
(1-3-12-14×12) 28-8	46	7.9 ±.04	.36
Combined	153	7.84±.021	.385
(3-2-4-1×13) 21-2	42	8.0 ±.04	.41
(3-2-4-1×13) 21-21	46	8.1 ±.03	.28
(3-2-4-1×13) 24-18	43	8.1 ±.04	.38
(3-2-4-1×13) 24-46	41	8.4 ±.03	.29
Combined	172	8.14±.019	.373
RECESSIVE PARENTAL			
1-3-12-14-2-23	28	1.9 ±.07	.57
1-3-12-14-2-25	26	2.3 ±.09	.67
Combined	54	2.10±.059	.646
3-2-4-1-22-1	26	2.1 ±.08	.63
3-2-4-1-22-27	25	1.8 ±.07	.49
Combined	51	1.97±.056	.590
RECESSIVE F_2			
(1-3-12-14×12) 20-19	42	1.0 ±.05	.52
(1-3-12-14×12) 20-24	37	1.1 ±.05	.46
(1-3-12-14×12) 28-32	48	2.0 ±.07	.68
(1-3-12-14×12) 28-49	47	1.5 ±.05	.50
Combined	174	1.45±.035	.690
(3-2-4-1×13) 21-16	36	2.1 ±.06	.55
(3-2-4-1×13) 21-22	47	0.9 ±.04	.41
(3-2-4-1×13) 24-25	34	2.0 ±.07	.62
(3-2-4-1×13) 24-48	29	2.0 ±.08	.67
Combined	146	1.66±.042	.747

SEGREGATION IN THE FIRST GENERATION

The data at hand indicate that there may have been a slight degree of segregation in F_1 although the evidence is not very conclusive, progenies of only two plants in each of the F_1 progenies having been grown. Table IX gives, for each cross, the grades of the F_1 parent individuals, the means of the spotted and spotless classes in their F_2 progenies and the means of the dominant and recessive F_3 populations derived from each F_1 individual. Each pair of plants selected in F_1 had differed by more than a half-grade. The differences between the corresponding F_2 progenies are in the same direction but are smaller than in F_1 and are not significant or barely significant. The differences between the F_3 populations are not only in the same direction as in F_1 and F_2 but seem to be significant in all but one case.

TABLE IX.—*Segregation in F_1 as indicated by comparison of the means of the spotted and the spotless plants in the F_2 progenies and of the means of the dominant and recessive F_3 populations descended from each F_1 plant*

F_1 progeny.	F_1 parent and F_2 progeny No.	Grade of the F_1 parent.	Means of the 2 classes in F_2.		Means of the F_3 populations.[b]	
			Spotted plants.[a]	Spotless plants.	Dominant.	Recessive.
1-3-12-14×12.....	20	7.0	6.71±0.076	0.44±0.050	7.79±0.031	1.06±0.037
1-3-12-14×14.....	28	7.6	6.96± .071	.70± .062	7.89± .028	1.78± .045
Differences...	.....	.6	.25± .104	.26± .080	.10± .042	.72± .058
3-2-4-1×13.....	21	7.2	6.90± .101	.64± .131	8.06± .026	1.42± .054
3-2-4-1×13.....	24	7.9	7.11± .092	.68± .078	8.23± .027	1.98± .055
Differences...	.....	.7	.21± .136	.04± .152	.17± .037	.56± .077

[a] Dominant and heterozygous plants. [b] Each F_3 population comprises two progenies, taken as one array

EVIDENCE OF MODIFYING FACTORS

The questions suggest themselves whether, in addition to the major factor determining full development of the spot as contrasted with its almost complete absence, there are minor factors which modify the degree of its expression; and whether such factors have been variously recombined in the hybrids.

No evidence of modifying factors is afforded by the results of selection during successive generations in the parental populations. In 1920, 1921, and 1922 the plant which gave the lowest average grade for petal spot in each of the recessive parental progenies was selfed and became the progenitor of a progeny grown the following year. Comparison of the parental values and the progeny means, as given in Table X, does not show a tendency to reduction of the spot. It will be shown presently that the apparent marked increase in the progenies grown in 1923 is probably attributable to the lateness of flowering of most of the plants in the parental progenies of that year.

In 1922 two plants were selfed in each of the parental progenies, these plants having represented the extremes of development of the spot for the population in question. Progenies of each of these plants were grown in 1923. The progeny means, as stated in Table XI, showed no differences of probable significance except in the third pair and in that case the higher mean was yielded by the progeny of the plant which had given the lower value.

TABLE X.—*Grade of petal spot of selections in the recessive parental populations and mean grade of the progenies of these selections*

Selection and year.	Grade of the selected individual.	Mean grade of its progeny grown the year following.
1-3-12, No. 14 (1920).....	0	1.2±0.11
1-3-12-14, No. 2 (1921).....	0.7	.7± .04
1-3-12-14-2, No. 25 (1922).....	.4	2.3± .09
3-2-4, No. 1 (1920).....	0	.7± .06
3-2-4-1, No. 22 (1921).....	.2	.7± .03
3-2-4-1-22, No. 27 (1922).....	.2	1.8± .07

TABLE XI.—*Petal spot grade of pairs of plants selected in each parental progeny of 1922 as representing the extremes of the progeny and means of the progenies of these plants grown in 1923*

Progeny and selection.	Petal spot grade of the individual selected in 1922.	Mean of its progeny grown in 1923.
SPOTTED PARENTAL FAMILIES		
12–13 No. 2	7.3	8.1±0.04
12–13 No. 23	7.9	8.0± .05
Difference	.6	.1± .064
13–8 No. 17	7.2	8.1± .04
13–8 No. 4	8.0	8.2± .04
Difference	.8	.1± .057
SPOTLESS PARENTAL FAMILIES		
1–3–12–14–2 No. 25	.4	2.3± .09
1–3–12–14–2 No. 23	1.2	1.9± .07
Difference	.8	.4± .114
3–2–4–1–22 No. 27	.2	1.8± .07
3–2–4–1–22 No. 1	.9	2.1± .08
Difference	.7	.3± .106

There are, however, indications of a recombination of modifying factors in the hybrids. Evidence has been presented that a slight degree of segregation took place in F_1 and it has been mentioned (p. 499) that in four pairs of F_3 progenies the means differed significantly, although each pair had the same F_1 grandparent. Nine of the 16 homozygous F_3 progenies, when compared with the population representing the respective dominant or recessive great grandparent (Table VIII) showed increase or diminution of the spot, the F_3 mean having differed from the parental mean by an amount equal to four or more times the probable error of the difference. The most pronounced of these differences are stated in Table XII, there being two cases in which dominant and three cases in which recessive F_3 progenies differed very significantly from the corresponding parental population. In one of the dominant F_3's the spot was increased and in the other it was diminished. All three of the recessive F_3's show a marked diminution of the spot, amounting in each case to a full grade.

This evidence from F_3 can not be accepted without reservation, however, for the reasons that soil heterogeneity of the plat used in 1923 resulted in considerable differences in the rate of development and earliness of flowering of the plants in the various progenies and that the degree of expression of the spot appears to have been affected by the stage of development of the plant.

The mean grade of petal spot was determined separately for the retarded and for the earlier-flowering plants in 11 recessive parental and F_3 progenies of 1923. In every progeny the more backward plants gave a higher mean grade than the more advanced plants, the average differ-

ence for all 11 progenies having amounted to 0.5 grade and the difference having been significant in 8 of the progenies. A similar comparison in 10 dominant parental and F_3 progenies showed no consistent tendency to greater development of the petal spot in the retarded as compared with the earlier-flowering plants.

TABLE XII.—*Evidence of increase or diminution of the petal spot in nonsegregating F_3 progenies as compared with populations representing the respective dominant or recessive parent of the cross*

Population.[a]	Mean grade of petal spot.	Difference between means, showing petal spot in F_3 as compared with parental population to have been—	
		Increased.	Diminished.
DOMINANTS			
Parental 13–8	8. 1±0. 028	0. 3±0. 042	
F_3 24–46	8. 4± . 031		
Parental 12–13	8. 1± . 032		0. 6±0. 064
F_3 20–14	7. 5± . 055		
RECESSIVES			
Parental 1–3–12–14–2	2. 1± . 059		1. 1± . 080
F_3 20–19	1. 0± . 054		
Parental 1–3–12–14–2	2. 1± . 059		1. 0± . 078
F_3 20–24	1. 1± . 051		
Parental 3–2–4–1–22	2. 0± . 056		1. 1± . 069
F_3 21–22	. 9± . 040		

[a] The parental population in each case comprises two progenies taken as one array.

As a measure of the comparative earliness of the several populations, the mean number of flowers per plant during the first four days of the grading period (July 14–17) was computed for each parental population and homozygous F_3 progeny. The correlation between the progeny means for number of early flowers and grade of petal spot was then computed separately for the 10 recessive and for the 10 dominant parental and F_3 populations. There was found to be a pronounced negative correlation in the case of the recessives, the coefficient having been $-.67\pm.10$. In other words, recessive progenies in which many of the plants were late in development tended to have a relatively high mean for petal spot. The dominant populations, on the other hand, showed an entire absence of correlation between earliness and grade of petal spot.

Both the dominant and the recessive parental populations, which were situated together at one end of the plat, proved to be inferior to most of the F_3 progenies in flower production at the beginning of the grading period. Many of the plants in the parental progenies did not begin to flower, or flowered very sparingly, until near the end of the grading period. Since, in the spotless populations, retardation of growth tends to a more pronounced expression of the spot, it is obvious that the means for petal spot grade of backward and of more advanced recessive popu-

lations are not fairly comparable. This objection applies to all comparisons of recessive F_3 progenies with recessive parental populations in Table XII, the former having had, in every case, a higher rate of early flowering than the latter. This factor of relative earliness of the plant also affects the comparisons between recessive F_3 progenies which had the same F_1 grandparent. Of the two pairs of recessive F_3's mentioned on page 499 as showing significant differences in mean grade of petal spot (28–32 and 28–49, 21–16 and 21–22), in each case the progeny which gave the lower mean for petal spot had the higher rate of early flowering.

On the other hand, the evidence of the occurrence of modifying factors from comparisons involving dominant F_3 progenies is not vitiated by differences in earliness, since in the dominant populations a retarded condition of the plants apparently did not result in a more pronounced expression of the petal spot. Of the two comparisons of dominants in Table XII, one (F_3 24–46) gave a significantly higher and the other (F_3 20–14) gave a significantly lower mean grade of petal spot than the corresponding dominant parental population, yet the rate of early flowering in both F_3's had resembled the parental rate. Comparison with one another of the dominant F_3 progenies 20–14 and 20–15, both of which had the same F_1 grandparent, shows that the latter gave a significantly higher mean for petal spot grade, although it had much the heavier rate of early flowering. The relations of earliness and development of the petal spot are in this case the reverse of what was noted in the recessive populations.

Returning to the indications of segregation in the first generation afforded by a correspondence between the differences in F_1 and F_3 (Table IX) it may be said that, in this case, the factor of relative earliness did not operate consistently in the recessive populations. In the cross 1–3–12–14 × 12 the recessive F_3 descendants of F_1 plant 28 gave a very significantly higher mean for petal spot than the recessive F_3 descendants of F_1 plant 20; and in the cross 3–2–4–1 × 13 the recessive F_3 descendants of F_1 plant 24 gave a very significantly higher mean for petal spot than the recessive F_3 descendants of F_1 plant 21. But the heavier rate of early flowering was shown in the first cross by the population which gave the higher mean for petal spot and in the second cross by the population which gave the lower mean for petal spot.

The position as regards modifying factors for petal spot in this material may be summed up in the statement that there are indications, but not cionclusive proof, of the existence of such factors which, by their segregation and recombination, have brought about a slight degree of differentation in the hybrids.

VARIATION IN PETAL SPOT ON THE INDIVIDUAL PLANT

The frequency distributions based upon averages of from 3 to 10 flowers per plant (Table VI) show a gap, amounting to 2.5 grades, between the plants which gave the lowest averages in the dominant parental and F_3 populations and the plants which gave the highest averages in the corresponding recessive populations. The gap is bridged, however, if individual flowers be considered. This is shown by the data presented in Table XIII, which gives the grades of the individual flowers grading lowest in the 12 dominant and highest in the 12 recessive parental and F_3 progenies of 1923.

TABLE XIII.—*Overlapping of the dominant and recessive populations of 1923 in grade of petal spot of the individual flowers*

Group.	Total number of flowers graded.	Numbers of flowers grading.		
		4.0	4.5	5.0
Dominants	3,843	9	3	15
Recessives	3,700	97	5	1

The average degree of variation on the individual plant may be shown by computing, for a given population, the mean of the differences between the flower graded highest and the flower graded lowest on each individual. In computing these means, plants were disregarded on which fewer than ten flowers were graded. The means were computed separately for the dominant and for the recessive parental and F_3 populations, each as one array, and for the dominant, heterozygous and recessive plants in the segregating F_3 progenies,[7] giving, in all, 5 populations of which the mean and maximum variation, on the individual plant, are stated in Table XIV.

TABLE XIV.—*Mean and maximum variation of petal spot grade on the individual plants in the dominant and recessive parental and F_3 populations and in the dominant, heterozygous and recessive classes of the segregating F_3 progenies.*

Population.	Number of plants.	Range of grades on the individual.	
		Mean.	Maximum and (in parenthesis) the number of plants showing it.
Dominant parental and F_3	297	2.10±0.035	5.0 (6)
Recessive parental and F_3	278	2.64± .026	5.0 (1)
Dominants in segregating F_3's	62	2.18± .060	4.5 (2)
Heterozygotes in segregating F_3's	107	2.55± .057	5.0 (3)
Recessives in segregating F_3's	66	2.42± .057	4.0 (2)

It is noteworthy that variation amounting to four or five grades is shown by a few individuals in each population. Comparison of the mean ranges shows significantly less variation in the dominant than in the recessive population. The dominants in the segregating F_3 progenies also are individually less variable than the heterozygotes and the recessives, although the difference between dominants and recessives is, in this case, slightly less than three times its probable error. The smaller variation of the dominants may be apparent rather than real, for the reason that slight differences are less easily detected when the spot is strongly developed than when it is weakly developed.

[7] Plants giving an average grade of 7.3 or higher were taken as dominant, 7.3 having been the lowest average given by any plant in the dominant parental populations. Plants giving an average grade lower than 7 and higher than 5 were taken as heterozygotes, the comparatively few individuals which averaged 7 to 7.2 having been left out of account as of uncertain classification. It is believed that classification on this basis is sufficiently accurate for the purpose in view. There could be no question as to the identity of the recessives.

The variation in grade of petal spot on the individual plant appears not to be due solely to variation in factors of the external environment. This is indicated by comparing flowers graded on the same plant on the same day. Of such comparisons, in 1923, 75 showed a difference of 2 grades, 31 of 2½ grades, 24 of 3 grades, 6 of 3½ grades, 2 of 4 grades and 3 of 5 grades. It may be argued, however, that since it is a rare occurrence for more than one flower to open on the same fruiting branch on the same day, these differences in the petal spot of flowers opening simultaneously on the same plant may be attributed to variations in the environment, either external or internal, during the period when the successive fruiting branches were being developed.

The F_1 hybrid progenies grown in 1921 and the F_2 hybrid progenies grown in 1922 did not show greater mean variation in petal spot on the individual plant than did the corresponding parental progenies. These facts, together with the absence of significantly greater individual variation in the heterozygous than in the recessive plants of the segregating F_3 progenies grown in 1923 (Table XIV) indicate that the type of "vegetative segregation" recently described by Gates [8,9] does not occur in this case.

RELATION OF PETAL SPOT TO SIZE OF THE FLOWER

It has been noted, in normal Pima populations, that the petal spot often is less well developed in the small and sometimes misshapen flowers borne by stunted plants than in the larger flowers borne by well-grown plants. The spotless families described in this paper showed no apparent inferiority to the spotted families in the vegetative vigor and fruitfulness of the plants, when grown under comparable conditions. It has been observed, however, in grading petal spot in the spotless populations that flowers showing no trace of the spot often have a small corolla and relatively few and small stamens, while flowers of normal size in the same populations often exhibit a faintly developed spot.

Comparison of the mean corolla length of spotted plants and of spotless plants in the F_2 progenies of 1922 showed a difference of only 1.5 ± 0.39 mm. in favor of the former, the mean length having been 60.4 ± 0.20 mm. in the spotted class (276 flowers) and 58.9 ± 0.33 mm. in the spotless class (123 flowers). In 1923 a dominant and a recessive F_3 progeny were compared, with negative results. The two progenies, descendants of the same F_1 plant, were situated in adjacent and conterminous rows, hence soil heterogeneity was not a factor. One corolla was measured on one plant in each progeny, 41 flowers having been measured in the dominant progeny and 42 flowers in the recessive progeny. The mean corolla length was 62.3 ± 0.36 in the former and 63.0 ± 0.39 in the latter, the difference amounting to only 1.3 times its probable error.

Although these comparisons of the mean corolla length of the spotted and of the spotless populations showed little or no difference, a significant positive correlation between grade of petal spot and length of corolla was found to exist within each class. In the F_2 progenies of 1922, 276 flowers of the spotted class on which both characters were measured

[8] GATES, R. RUGGLES. VEGETATIVE SEGREGATION IN A HYBRID RACE. *In* Jour. Genetics, v. 6, p. 237-253, pl. 9. 1917. List of references, p. 252-253.

[9] ——— A PECULIAR TYPE OF VARIABILITY IN PLANTS. *In* Jour. Genetics, v. 13, p. 13-45, 24 fig. 1923. Literature cited, p. 44-45.

yielded a coefficient of correlation of $0.645 \pm .024$ and 123 flowers of the spotless class gave a coefficient of $0.307 \pm .055$.[10]

ENVIRONMENTAL REACTIONS OF THE PETAL SPOT

There is good reason to believe that both the size and the intensity of color of the petal spot vary in response to variations in the physical environment, although this has not been tested by controlled experiments. Soil factors play a part, not only indirectly, by affecting the rate of development of the plant (p. 502) but probably in a more direct manner. Thus it has been observed that in Pima cotton fields the petal spot is less well developed where the plants are stunted than where they have made a normal growth, although the same stock of seed was used in planting the entire field. It has been shown that a positive correlation exists between size of flower and development of the spot, and since the flowers borne by stunted plants usually are small, the comparatively weak development of the spot on such plants may be regarded as part of a general reduction caused by the unfavorable environment.

The influence of meterological factors will be examined, first, by comparing the means for petal spot in different years and, second, by comparing the earlier and the later flowers produced by the same individual plant during the same season.

Data given in Table I indicate that conditions in some years favor a more pronounced expression of the petal spot, the mean grade in both spotted and spotless populations, but especially the latter, having been notably higher in 1923 than in other years. It was pointed out, in discussing the evidence for the occurrence of modifying factors, that the lateness of flowering of the plants in the spotless parental progenies of 1923 may have been a factor contributing to the relatively high mean of that year. Yet the more pronounced development of the spot in 1923 can not be attributed wholly to this factor, for the spotted parental population, in which the backward plants did not seem to have the spot increased as was the case in the spotless population, also gave a significantly higher mean than in previous years. Comparison of the spotless populations of successive years in respect to the percentages of flowers showing no trace of the spot gives further evidence that conditions in 1923 favored an exceptionally high degree of expression of the spot. Because of the retarded growth of most of the plants in the parental populations of 1923, the recessive F_3 progenies, in which most of the plants had shown a normal rate of growth, were compared with the parental populations of the two preceding years. The percentages of entirely spotless flowers were as follows, the figures in parenthesis indicating the total numbers of flowers graded: 1921 (290) 43.8 per cent; 1922 (561), 45.6 per cent; 1923 (2,826), 16.1 per cent.

In endeavoring to ascertain whether the degree of expression of the petal spot differs in different periods of the same season, comparison was made in 1922 of the means based upon the first 5 flowers and the second 5 flowers graded on the same individual plants. The two means were computed separately for 143 dominant plants and for 85 recessive plants.

[10] Corolla length and petal spot were correlated positively and significantly in a hybrid between upland and Egyptian cottons, the coefficient of correlation for 180 F_3 plants having been $0.244 \pm .047$. The correlations of petal spot with 37 other characters were determined on this material and in all but two cases the coefficient of correlation was less than 3.5 times its probable error. The correlations in question were with corolla length and with calyx dentation, r in the latter case having been $-.220 \pm .048$. KEARNEY, Thomas H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Bul. 1164, p. 45, Table 12. 1923.

The means for the dominant plants showed no difference between the earlier and the later flowers but, for the recessive plants, the mean for the earlier flowers was $0.24 \pm .039$ grade higher than the mean for the later flowers. A similar comparison in 1923 on 285 dominant and 267 recessive plants on which 10 flowers per plant had been graded showed, in both groups, a difference of 0.1 grade in favor of the earlier flowers, the difference in one case having been 6 and in the other case $4\frac{1}{2}$ times its probable error. In both years, therefore, comparison of the earlier with the later flowers on the same plants gave evidence of a mathematically significant but practically unimportant difference in grade of petal spot in favor of the earlier flowers.

The fact that the early, as compared with the later flowers produced by the same individual, tend to give a slightly higher average grade of petal spot seems at first glance inconsistent with the fact previously noted (p. 502) that the petal spot is apt to be better developed on retarded plants than on plants which have grown more rapidly. The apparent contradiction disappears if we consider that the earlier flowers on the more advanced plants were produced when these plants were in a stage of growth more nearly comparable to that of the retarded plants.

A more marked reduction in the petal spot takes place towards the end of the flowering season. On October 6, 1923, it was noted that in both the dominant and recessive populations the flowers showed a pronounced diminution in the size and intensity of the spot as compared with the condition on August 18, when the grading ended.

FREQUENCY OF 4-LOCK BOLLS IN RELATION TO PETAL SPOT

Observation of the growing plants and measurement of the leaves, bolls and fiber have shown that the families which furnished the spotless parents of the hybrids here described are typical Pima except in the development of the petal spot and the proportion of 4-lock bolls. In other color characters of the flowers, greenish yellow color of the petals and empire yellow color of the pollen, they show no departure from the type. In percentage of 4-lock bolls, the descendants of both of the spotless selections of 1917 exceed the average for the variety as grown at Sacaton. This was shown by data from the progenies grown in 1919 and 1920, in comparison with the general stock of Pima cotton.[11] Additional evidence was obtained in 1921 and 1922, by comparing the spotless parental progenies with progenies representing the normal or spotted parents of the hybrids. Data for both the parental and the hybrid populations are given in Table XV.

It is clear from the data in Table XV that in 1921 and 1922 the progenies representing the two spotless families had significantly higher mean percentages of 4-lock bolls than the progenies descended from the spotted parents of the crosses, just as, in 1919 and 1920, the spotless families had significantly higher percentages than the "bulk" Pima with which they were compared. It is also evident that the descendants of spotless plant No. 3 continue to give very significantly higher means for this character than the descendants of spotless plant No. 1. In F_1 the percentage of 4-lock bolls in one of the crosses resembles that of the progeny representing its spotless parent, while the other cross gave an almost intermediate percentage. In F_2, the mean percentage of 4-lock

[11] KEARNEY, Thomas H. HERITABLE VARIATIONS IN AN APPARENTLY UNIFORM VARIETY OF COTTON. *In* Jour. Agr. Research, v. 21, p. 237–241. 1921.

bolls in both crosses is somewhat below the average of the parental means, but is significantly higher than the percentage of the normal or spotted parent. The heritability of this character is shown further by the fact that in F_2 the cross involving 3–2–4, the spotless family having the higher percentage of 4-lock bolls, gave a mean percentage of 4-lock bolls nearly twice as great as the cross involving spotless family 1–3–12. Expressed as a percentage of the lower mean, the difference between the hybrids (F_2) amounted to 89 per cent while the difference between the spotless parental progenies grown the same year (1922) amounted to 131 per cent.

TABLE XV.—*Percentages of 4-lock bolls in the parental and hybrid populations grown in 1921 and 1922*

Populations of 1921.	Number of plants.	Mean percentage, 4-lock bolls.	Populations of 1922.	Number of plants.	Mean percentage, 4-lock bolls.
PARENTAL			PARENTAL		
1–3–12–14 (spotless)...	14	4. 15±0. 34	1–3–12–14–2 (spotless)	22	4. 54±0. 37
12 (spotted).....	20	2. 45± . 21	12–13 (spotted).....	19	1. 84± . 15
Difference.....	.....	1. 70± . 40	Difference.....	.....	2. 70± . 40
HYBRID (F_1)			HYBRID (F_2)		
1–3–12–14×12.....	28	4. 50± . 28	1–3–12–14×12[a].....	83	2. 71± . 13
PARENTAL			PARENTAL		
3–2–4–1 (spotless).....	27	9. 31± . 33	3–2–4–1–22 (spotless).	26	10. 50± . 53
13 (spotted).....	14	2. 92± . 24	13–8 (spotted).....	22	2. 44± . 24
Difference.....	.....	6. 39± . 41	Difference.....	.....	8. 06± . 58
HYBRID (F_1)			HYBRID (F_2)		
3–2–4–1×13.....	33	5. 87± . 30	3–2–4–1×13[a].....	62	5. 13± . 23

[a] Two progenies as one array.

There appears to be no general correlation, either negative or positive, between the degree of development of the petal spot and the mean number of boll locks per plant. In F_1 and F_2 of a hybrid between upland and Egyptian cottons, the coefficients of correlation for this pair of characters were, respectively, -0.043 ± 0.137 and 0.011 ± 0.050. As concerns the material dealt with in this paper, the coefficient of correlation for 125 plants in the progenies of the spotless families grown in 1920 was 0.069 ± 0.060. The correlations were also worked out for the spotted (dominant and heterozygous) plants in the F_2 progenies of 1922, taking as one array in each case the two progenies of the cross 1–3–12–14×12 and the two progenies of the cross 3–2–4–1×13. The numbers of plants were, respectively, 55 and 48 and the coefficients of correlation obtained were -0.045 ± 0.091 and 0.148 ± 0.095.[12] A further indication of the absence of linkage was afforded by comparison of the mean percentages of 4-lock bolls, of the spotted and the spotless segregates in each F_2 progeny. There was in no case a significant difference between the two

[12] The correlations worked out in the upland×Egyptian hybrid were between petal spot grade and mean number of boll locks per plant, while in the crosses described in this paper the correlations were between petal spot grade and percentage of 4-lock bolls. These are merely different expressions of the same relation, practically all of the bolls in this variety of cotton having either three or four locks.

means. Finally, the percentage of 4-lock bolls was determined in 1923 for the homozygous F_3 progenies 21–21 and 21–22, the former dominant and the latter recessive for petal spot. Soil heterogeneity was not a factor in this comparison, the two progenies having been grown in adjacent and conterminous rows. The percentages were 5.11 ± 0.33 and 5.33 ± 0.34, the difference having been much smaller than its probable error.

The two plants of 1917 which became the progenitors of the two spotless families were selected late in the fall in the course of a search for plants having exceptionally numerous 4-lock bolls. The unusual character of their flowers was first noticed in their progenies. The spotless character is extremely rare in the Pima variety and, indeed, has not been observed except in the descendants of these individuals. Considering this fact and the absence of linkage between spotless petal and a high percentage of 4-lock bolls, the coincidence would be an extraordinary one if the original spotless plants had been unrelated. It is much more probable that, although discovered almost by accident in a field grown from "bulk" seed, they were both descended from an individual presenting a chance combination of the two characters.

DISCUSSION

Comparatively few characters of the cotton plant have been proved conclusively to segregate in definite Mendelian fashion. It has seemed worth while, therefore, to present rather fully the evidence that the character petal spot affords an example of such behavior. The sharpness of the segregation in this case is interesting because the recessive allelomorph usually is expressed in a faintly spotted rather than an absolutely spotless condition. It is also of interest that the character proves to be decidedly variable in the homozygous populations, whether recessive or dominant, variation being shown not only from plant to plant and in response to environic influences, but among flowers produced simultaneously on the same individual plant.

Petal spot, unlike such characters as fruitfulness, size of the boll and length and abundance of the fiber, is of no direct agricultural or commercial importance. Nevertheless, any character which may serve as the "hall mark" of an agriculturally desirable strain or variety is likely to prove useful. This is particularly the case in a crop like cotton, since the commercial value of the product depends largely upon its uniformity and this, in turn, upon the genetic purity of the stock. Cotton is subject to cross-pollination and, consequently, in order to maintain pure planting seed of a variety, it is often necessary to rogue seed-increase fields which have been grown in proximity to another variety or stock, in order to eliminate accidental hybrids. It is obvious that roguing can be done thoroughly only if the stock to be rogued possesses characters which make it easy to distinguish the hybrids resulting from cross-pollination with other stocks. It is also important that the distinguishing characters be recognizable early in the summer, before there has been opportunity for extensive cross-pollination of the parent stock by the hybrids present in the same field.

The Pima variety is so uniform morphologically that it is usually impossible to detect, in time for roguing, the offspring of accidental crosses between strains which have been selected on the basis of fruitfulness, earliness and properties of the fiber and seeds. Such a character as spotless petal meets all the requirements of a serviceable "hall mark" for its

allelomorph, full-spotted, is almost completely dominant. Consequently the first-generation heterozygotes resulting from accidental cross-pollination with a normal stock of the variety could be recognized as soon as the first flowers open.

Since spotless petal is a simple recessive, there should be no great difficulty in transferring this character to any other strain of the Pima variety. Complications would ensue were spotless associated with marked inferiority in characters of practical importance. Fortunately, the spotless families described in this paper are satisfactory in fruitfulness and in the length, strength, and abundance of the fiber, hence there is no reason to expect that the offspring of such a cross would be inferior, either agriculturally or commercially.

SUMMARY

A conspicuous red spot near the base of the petal characterizes Sea Island and Egyptian cottons, while the petals of most varieties of upland cotton are spotless. In a hybrid between the Holdon variety of upland cotton and the Pima variety of Egyptian cotton, this character showed unifactorial segregation, the ratio of spotted to spotless plants in F_2 having been approximately 3:1.

Two plants, discovered in 1917 in a field of Pima cotton, gave rise to families breeding true for a spotless or very faintly spotted petal. Crosses between these families and an inbred family in which the spot is fully developed gave opportunity for study of the inheritance of this character within the Pima variety.

In the first generation of the hybrid the spot was almost but not quite dominant, the hybrid means having been significantly lower than those of the spotted parental populations. The variability of the hybrid was of the same order of magnitude as that of the parental families.

The second generation of the crosses showed very definite segregation into a spotted and a spotless (strictly speaking a very faintly spotted) class, the percentages of spotless individuals not departing significantly from the 25 per cent expected with a single factor difference. The variability within the spotted class was, however, greater than in the populations representing the spotted grandparents, and in two of the four F_2 progenies the frequency distributions of the spotted segregates were bimodal. These facts indicate that the heterozygotes are partly distinguishable from the pure dominants.

In the third generation 24 progenies were grown from plants in each F_2 progeny representing, respectively, the most spotted, least spotted and most nearly intermediate condition. The behavior in F_3 was in complete accord with the expectation. The eight progenies of fully spotted F_2 plants bred true, as did the eight progenies of spotless F_2 plants, while there was sharp segregation in each of the eight progenies of F_2 plants which were most nearly intermediate. The percentages of spotless individuals in the segregating F_3 progenies in no case departed significantly from the expected 25 per cent. When all of the segregating progenies are taken as one array the percentage of spotless individuals is 26.5 ± 1.6. The proof is conclusive that the spotless or very faintly spotted condition is a simple recessive.

The spotted class in seven of the eight segregating F_3 progenies gave a bimodal distribution, this fact confirming the evidence from F_1 and F_2 that full-spotted is not completely dominant and that the heterozygotes are partly distinguishable.

The question whether modifying factors are involved, in addition to the major factor which determines presence of the spot as contrasted with its complete or nearly complete absence, was not answered decisively. There were indications of a slight degree of segregation in F_1 and some of the dominant and recessive F_2 progenies showed apparently significant increase or diminution of the spot in comparison with the corresponding parental population. But it was found that environmental factors had contributed to these differences in F_2 by influencing the rate of development of the plants in the several populations. Furthermore, selection within the parental lines appears to have resulted in no perceptible modification of the spot. Yet, although the evidence at hand does not prove the existence of modifiers, it by no means justifies the conclusion that such factors are not involved.

The character petal spot shows considerable variation on the individual plant, even among flowers which open on the same day. The variation was not greater in the heterozygous than in the homozygous populations.

The degree of expression of the petal spot was found to be correlated positively and significantly with size of the flower, in both spotted and spotless populations. There was, however, no evidence of linkage.

The spot is affected by the environment, being responsive to soil variations and also to seasonal changes. Differences were noted both as between different seasons and as between different periods of the same season.

The spotless families are typical Pima in all other characters, except that they have given consistently a higher proportion of 4-lock bolls than normally spotted Pima populations grown under comparable conditions at the Sacaton Station.

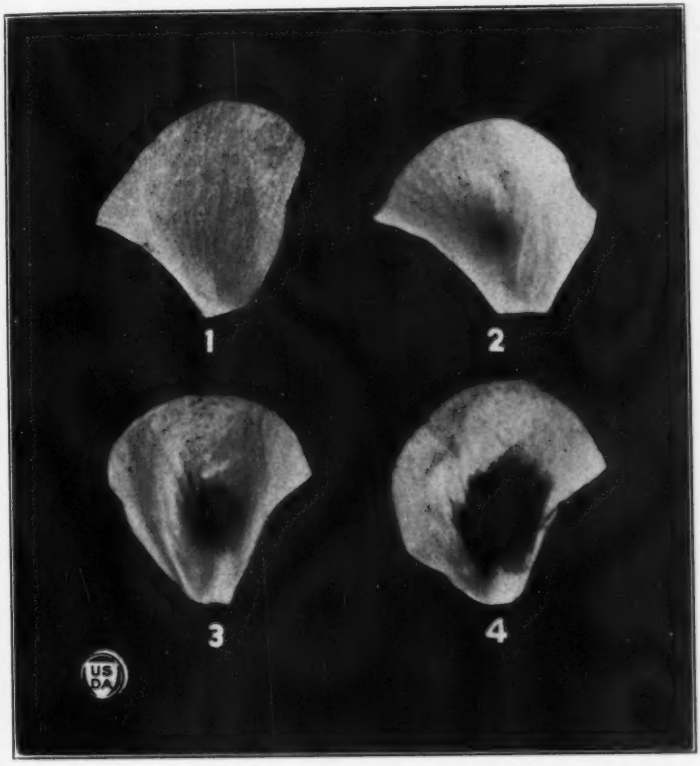
There is an entire absence of linkage between spotless petal and a relatively high mean number of boll-locks and the spotless condition has been observed only in the two families described in this paper. It is probable, therefore, that the progenitors of these families, although discovered in a population grown from "bulk" seed, were nearly related if not sister plants.

Such a character as spotless petal should be useful as the "hall-mark" of an agriculturally and commercially valuable stock of Pima cotton. The fact that spotless is recessive would make it easy, in roguing seed-increase fields of a strain possessing this character, to recognize the first generation hybrids resulting from accidental cross-pollination with normal Pima, since they would have the spot well developed.

The recessive nature of spotless should make it readily transferable by hybridization to any desirable strain of this variety. No unfavorable results from such combinations need be anticipated, for the existing spotless families are not inferior to the average of the variety in fruitfulness and in the properties of the fiber.

PLATE 1

Basal portions of petals of Pima cotton showing the range of grades in the spotless and normal families and in the hybrid between them. Grades 0 (fig. 1) and 3 (fig. 2) represent approximately the extremes of the recessive populations, while grades 6 (fig. 3) and 9 (fig. 4) represent approximately the extremes of the dominant populations.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THE MIGRATION OF THE APHIDIDAE AND THE APPEARANCE OF THE SEXUAL FORMS AS AFFECTED BY THE RELATIVE LENGTH OF DAILY LIGHT EXPOSURE[1]

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INTRODUCTION

It is generally stated in textbooks that on the setting in of cold weather there is produced in the aphids a generation of males and females. Temperature has been considered to be the determining factor in the appearance of the sexual forms. As to the factors which influence the early or late production of migratory forms, Matheson (*15*)[2] remarks that suggestions have not been made by any worker.

During the course of the investigation of the strawberry root louse (*Aphis forbesi* Weed), in 1921, it was observed that large numbers of the sexes made their appearance in the laboratory on November 3, this being somewhat earlier than they appeared in the field. At this time of the year the air in the laboratory is warmer than that outside, so that the temperature factor did not seem to be the cause of their appearance. A copy of Garner and Allard's (*6*) work came to the writer's attention just at this time, showing that flowering and fruiting of plants can be attained in certain varieties and species only when they are exposed to definite amounts of daily light such as obtain during certain seasons of the year. Plants and plant lice have this in common that sexual forms make their appearance only at certain definite periods; so it occurred to the writer that length of day was a factor which might influence the latter as well as the former.

A word of explanation in regard to the life-history of plant lice may be in place here because of their peculiar mode of development. There are many variations and modifications in the different species, but in general it may be stated that the winter is passed in the egg stage, especially in the more northern latitudes. In Tennessee the eggs hatch in the spring as early as February 12 into a form known as the stem-mother, which matures in from 8 to 14 days. The stem-mother is always wingless and gives birth to living young parthenogenetically—that is, without the presence of males. This mode of reproduction continues until the fall, when the true males and females make their appearance. Upon mating, the eggs are deposited which carry the insect through the winter. There are many variations in the general life cycle as just stated. The second generation, that is, the progeny of the stem-mother, may consist mostly of winged forms which migrate to other plants of the same species or even to entirely different species (secondary host) widely separated from the original host. The migratory forms may also make their appearance in any subsequent generation. After several genera-

[1] Accepted for publication, Jan. 2, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 522.

tions are produced on the secondary host, and with the approach of autumn, a winged generation is again produced that flies back to the original, or primary, host, where the true sexes are produced. In place of the migatory form to a secondary host there is sometimes produced a summer resting stage, or dimorph, as in the boxelder aphid.

DISCUSSION OF THE LITERATURE

A glance at the literature indicates that many explanations have been offered for this autumnal appearance of the fall migrants and the sexes, such as a failing food supply, adverse conditions, temperature, and seasonal conditions. Just what the seasonal factor is, such as the length of day, has not been discovered, and it is interesting to note that the literature itself records the appearance of the sexes earlier in the fall in the more northern latitudes, where the shortness of the day is more pronounced.

Smith (*16, p. 43–44*) in working with the clover aphis (*Anuraphis bakeri*), noted in Idaho that the "fall migrants were found on fruit trees on August 21, and by September 2 they were numerous. Yet the mean temperature for August in this locality was 69° F., which was the same as the mean temperature for July and 4.5° F. above the mean temperature for June." Smith further remarks that "temperature changes incident to the approach of winter may not be the chief factor serving to stimulate the development of fall migrants. As further evidence in this connection, clover plants artificially infested in late summer and transferred to warm rooms in the county courthouse at Twin Falls, developed males and winged females in great abundance during the following February and March."

TABLE I.—*Mean temperature records and length of day for Knoxville, Tenn., and the northern part of Scotland*

	Knoxville, Tenn., latitude 36°.		Sutherland, Scotland, latitude 58° 18'.	
	Temperature.	Length of day.	Temperature.	Length of day.
	° F.	*Hours.*	° F.	*Hours.*
January	38.8	10.0	38	7.1
February	41.9	10.9	38	9.4
March	48.7	11.9	40	11.7
April	58.0	13.1	44	14.4
May	67.2	14.1	48	16.7
June	74.0	14.6	53	18.2
July	77.1	14.4	56	17.6
August	74.7	13.5	56	15.4
September	70.6	12.4	52	12.9
October	59.9	11.3	48	10.4
November	47.9	10.2	42	7.9
December	40.3	9.7	39	6.4

Similarly, Jackson (*12*) records the presence of oviparous females and eggs of several species in Scotland during the first part of September. No doubt the sexes were already present in August if eggs were found early in September. A study of the temperature records for Scotland, as summarized in Table I, shows that July and August have about the same mean temperature of 56° F. On the other hand, a study of the length of

day for the northern part of Scotland reveals a drop of 4 hours from the long days of 18.2 hours in early July to 14.2 hours in the latter part of August.

Several species of plant lice, such as the spring grain aphis, cabbage aphis, and others, are known to pass the winter in the egg stage in the more northern climates, whereas farther south reproduction takes place throughout the winter without the presence of sexes. Such being the case, one is tempted to conclude that the northern temperatures stimulate the formation of the sexes; yet Baerg (*1*), working with *Myzus brevipilosus*, found that no sexes were produced in Arkansas, but when taken to New York sexes were produced abundantly in a warm laboratory in the winter. In this connection it should be remarked that along with the higher temperatures of the more southern latitudes the variation in the length of day is not so striking, and evidently not sufficient to stimulate egg production.

An extended study of the appearance of the sexual forms was made by Davidson (*5*), working with *Aphis rumicis*. He remarks that "on the approach of autumn, the influence of some important factors results in the development of sexual forms irrespective of the number of generations. There would appear to be at work some inherent tendency associated with adaptation to seasonal conditions. As autumn advances, an increased number of sexual forms appear." He concludes that "sexual forms develop under favorable food and temperature," and that "they were produced under the low temperatures of October in the glass-house, and later under the warm temperature in the greenhouse. Further, agamic and sexual forms appeared together under the same conditions of food and temperature. The autumn seasonal factor would appear to be important in accounting for the large number of sexual forms produced at this period." It is evident that Davidson recognized an autumnal factor, and had he used the factor of the length of day, his results would doubtless have been more in harmony with the facts.

It is generally recognized that the same insect differs in habits in different sections, necessitating different methods of control, but in some cases no adequate reason has been given. Lathrop (*13*) discovered that the apple aphids in Oregon differed considerably in their habits from those in New York. Hatching of the eggs of *Aphis sorbi* begins in Oregon about March 20, and is over a month earlier than in New York. The spring migrants leave for plantain in New York regularly in the third generation, and sometimes in the fourth, whereas, in Oregon the migration is drawn out and tends to be irregular. The fact observed by Baker and Turner (*2*), that migrants appear in increasing numbers up to the eighth generation in Virginia, which is further south, and where the length of day is less variable, would indicate that length of day, in relation to the time of hatching, is the determining factor. At the time the eggs hatch in Oregon the days are still short and do not offer the stimulus for migration. Furthermore, Lathrop's ability to keep *Aphis sorbi* on apple until November, by shading with cheesecloth, and the writer's experience in rearing 10 generations on apple by June, with the possibility of a still greater number, emphasize the importance of light in the migration of aphids.

The importance of knowing the factors that influence the production of migratory forms is clearly evident with *Aphis sorbi*, for should the fourth generation be produced on apple before migrating, extreme

injury might result. Matheson (*15*) records such a case in New York, where in 1915 "for some unknown reason scarcely any of these (the third generation) produced winged forms, and as a result the trees became so infested that scarcely a leaf or fruit could be found that was not crowded with lice. If the factors involved in the early or late production of the winged migrants could be determined, this would be a long step in solving some problems in insect control."

Jackson (*11, p. 541*) in a recent article takes up the effects of nutrition on morphology and cites several early workers, such as Kyber (1813), Keller, and Behr, who noted the appearance of winged male forms in aphids as a result of underfeeding. These authors do not go into detail in regard to the methods used. Of course the giving of a short day to a plant would be a method of underfeeding.

EXPERIMENTAL DATA

INFLUENCE OF THE SHORT DAY ON THE PRODUCTION OF SEX FORMS

The plants used in the experiments were kept in pots and exposed to from 7½ to 8 hours of light by being placed in a dark, ventilated chamber, the door of which swings open from the top. The whole was kept in a screen-wire cage out of doors, where the temperature would not be materially different from that under natural conditions. The method used to subject the plants to a short day was to place the potted strawberry plants with the lice in the dark chamber at 5 o'clock in the afternoon. The following morning at 9.30 the plants were removed and placed in the light. Garner and Allard (*6*) showed in their experiments that the difference in temperature inside and outside the dark house was negligible, since the temperature inside was but 2° or 3° F. higher than the temperature outside; hence any responses on the part of the plants could not be attributed to lower temperatures. Single potted plants were also darkened by having inverted over them a larger pot or a box.

Aphis forbesi Weed.—The first preliminary experiment was conducted with this species which is known as the strawberry root louse. On May 23, 1922, lice were placed on a potted strawberry plant, which was then given a short daily light exposure of 7½ hours. By September 18, males and oviparous females were present in large numbers. The first eggs were laid September 22, whereas normally the first eggs are laid in the field early in November. In Tennessee, egg laying continues, under natural conditions, through the month of December.

In 1923, it was planned to start shortening the day to 7½ hours as soon as the eggs were hatching, which began on February 16. From February 23, the plants bearing the aphids were subjected to the short day. The first oviparous females were observed on May 7, and upon dissection yielded eggs. On May 22, eggs were deposited on the pedicels of the strawberry.

Another plant taken from the greenhouse with aphids in the second generation was subjected to a short day, starting March 21, and by May 12 oviparous females made their appearance.

Aphis pomi DeGeer.—The green apple aphis passes its entire life cycle on the apple. The eggs hatched March 13, on a small apple tree under observation, two weeks before the buds burst, so that all of the young perished out of doors. A few of the eggs were saved, and,

upon hatching, the young were placed on a small apple seedling in the dark chamber. The second generation appeared on April 13, entirely wingless, contrary to the habits of this species in the North. The first winged forms were observed on April 24. In spite of the fact that this species was kept on a short day until June 1, the sexes had not made their appearance. Several times they had increased to such an extent as to kill the plants. This necessitated the saving of a few stragglers that managed to live and the placing of them on a new plant which had not been exposed to a short day. The process of changing plants continued three times, which may account for the nonappearance of the sexes. The same was true with *Aphis spiroecola*.

Chaitophorus negundinis Thom.—The boxelder aphis is rather peculiar in that it has a resting stage, or dimorph, during the summer months. Winged forms are produced that fly to other boxelder (*Acer negundo*) trees, but as the summer approaches the dimorphs appear as the normal individuals disappear. With the approach of autumn the dimorphs become active and give birth to those forms which produce the sexes.

Experiments were undertaken to determine whether the dimorph is also a product of long days. Plants were kept under a long day of 15 hours, starting March 30, 1923. The eggs hatched out of doors on March 1. The first dimorphs were found out of doors on May 3, about the same time that they appeared in the long-day experiment. In a few weeks' time the aphids killed the plant kept in the dark chamber, so that the experiment had to be discontinued.

On May 20, 1923, a plant bearing dimorphs was placed in a dark room, and within a day the dimorphs started to move around, indicating their sensitiveness to light. Their reactions to a long and a short day, however, remain to be proved.

Aphid on Heuchera americana.—G. G. Ainslie, of the Bureau of Entomology, United States Department of Agriculture, called the writer's attention to a Macrosiphumlike aphid on *Heuchera americana* that produced the sex forms in April and deposited eggs in May, a procedure quite out of the ordinary. Here was a case so different that it was thought worth while to note the effect of light exposure on this species. Accordingly plants were placed in a dark chamber, as well as under an artificial long day, on March 27, when the stem-mothers were found producing young. Males and females made their appearance in both cases the first week in May, indicating that the sequence of generations has been determined from the egg stage onward.

INFLUENCE OF THE LONG DAY ON THE INHIBITION OF THE SEX FORMS.

From the experiments with *Aphis forbesi*, it is apparent that the production of the true sexes appears to be governed by the short days, such as are present during the fall of the year. If such is the case, it should be possible to keep the sexes from making their appearance by exposing the plants to a long day. Beginning with September 4, strawberry plants bearing *Aphis forbesi* were given 15 hours of daily light exposure by means of a 60-watt Edison Mazda light that hung about 2 feet from the plants. The plants were kept lighted out of doors, so as to keep the temperature factor constant. The use of the light did not alter the temperature near the strawberry plants. At the same time a control plant was placed inside of the laboratory window.

By October 6, the oviparous females were not present on the plants kept under the long-day conditions, although they were already present on the control plant. Examined December 3, the viviparous forms were still present out of doors on the strawberry plants receiving the long daily exposure.

INFLUENCE OF THE SHORT DAY ON MIGRATION

Aphis rumicis L—Having been successful in securing the sex forms by the use of a short day, and noting that in the migrations of plant lice summer is the time for dispersal to the secondary hosts, and that the return migrants make their appearance during the short days of the fall, the writer started a series of experiments to prove, if possible, that the migration of aphids is also influenced by the length of day. Several well-known migratory aphids were selected, such as *A. rumicis*, *Capitophorous hippophaes*, and *A. sorbi*.

In Tennessee the common snowball (*Viburnum opulous*) is the primary host of *A. rumicis*, and several weeds such as burdock (*Arctium lappa*) and curled dock (*Rumex crispus*) serve as the secondary, or summer, hosts.

The eggs began hatching on March 9, 1923, and small potted snowballs bearing *A. rumicis* were subjected to 7½ hours of daylight. The stem-mothers became full-grown April 2, while pupae made their appearance on April 16. These became winged on April 17, and represented the spring migrants. In this case, as with other species, the length of day is apparently not operative when certain stages are predetermined, and follow in quick succession. Most of the second generation, under normal conditions, become winged and migrate, and the production of migrants can not be stopped in so short a time.

On April 17 spring migrants were placed on curled dock (*Rumex crispus*), which was placed in the dark chamber and given about 7½ hours of light. The first males and sexuparae made their appearance on June 6. The sexuparae were then placed on snowball, where the oviparous females became mature June 20, at the hottest time of the year, and when the days are longest. In 1922, under natural conditions, the males, females, and sexuparae were present on snowball October 19.

Capitophorous hippophoes Koch.—This species, as determined by Gillette (*8*), winters on the Russian olive (*Eloeagnus umbullata*), and goes to smartweed (Polygonum sp.) for the summer months. The eggs began hatching February 27, 1923, on the Russian olive. A young plant with lice was placed in the dark chamber, and the first migrants made their appearance on April 10. These migrants were then placed on smartweed, which was transferred to the dark chamber to be subjected to the short day. The first generation became mature on smartweed April 25, and on May 24 the young of the sexuparae and males were first observed with the characteristic red dorsal stripe that is present on no other generation. The first males matured June 2, whereas normally they do not make their appearance until the latter part of October.

Gillette (*8*) records that in Colorado the second generation on Eloeagnus all acquire wings. Here in Tennessee most of the second generation acquired wings, but a few remained on the primary host as late as June. It is interesting to note that young smartweed growing in the greenhouse was in flower on March 26, when it was but 2 inches high.

Aphis sorbi Kaltenbach.—The rosy-apple aphis has for its alternate host the common narrow-leaved plantain (*Plantago lanceolata*). On

March 26, 1923, the eggs began to hatch and the stem-mothers matured about April 5, inside the laboratory, under a long day of 15 hours of artificial light. The second generation matured April 16, and was wingless. Winged forms, consisting of the third generation, appeared in large numbers on April 24 on the plant kept under a long day, whereas under similar temperature and humidity conditions, only two winged forms appeared in the third generation on an apple seedling kept in the laboratory away from artificial light.

Similarly 10 generations were reared on apple by June 12, without more than two or three winged forms appearing in each generation, simply by keeping the seedlings indoors under subdued light growing in plain tap water and giving each generation a new plant. These seedlings were often yellowish and weak-looking, because of lack of nourishment, and yet the winged forms appeared sparingly. When the seedlings were allowed to become crowded, however, and under similar conditions, the winged forms became very numerous, so that it appears as if crowding were a factor that induces the formation of winged forms. No doubt many more than 10 generations could have been reared on apple, but because of the writer's absence from the office the plants died.

Beginning with April 24, a narrow-leaved plantain with migrants was placed in the dark chamber. Winged forms that may have been summer migrants appeared as early as May 21. On June 6 winged forms from plantain kept in the dark chamber were placed on apple. They fed and deposited young, which lived on apple six days. Baker (3) has determined that with certain species the oviparous forms require hard, dry leaves to mature on, such as appear in autumn when the leaves are falling. It is very likely that for this reason the young oviparous females failed to mature, since all the apple leaves are very succulent in May. The appearance of the sexual forms is summarized in Table II.

TABLE II.—*Summary of the appearance of the sexual forms when subjected to a short day*

	Date of eggs hatching.	Date of transfer to dark chamber.	Date of appearance of sexual forms.	Date of normal appearance of sexual forms.
Aphis forbesi	Feb. 16	Feb. 23	May 7	Oct. 20 to Nov. 15.
Aphis forbesi	...do....	Mar. 21	May 12	Do.
Aphis rumicis	Mar. 9	Apr. 17 (on curled dock).	June 6	October.
Capitophorus hippophaes.	Feb. 27	Apr. 10 (on smartweed).	June 2	Do.
Aphis sorbi	Mar. 26	Apr. 24 (on plantain).	June 12	October to November.

Aphis viburnicola Gill.—This species passes the winter on the common snowball (*Viburnum opulus*), but its alternate host has so far not been discovered. The aphid occurs in Tennessee, although it is not common. Eggs received through the kindness of Professors Gillette and Ruggles hatched on March 12. The stem-mothers became full-grown March 26, and it was not until April 16 that they became full-grown out of doors. It is generally stated in the literature that all of the second generation acquire their wings and leave the snowball. With young snowball plants kept under a long-day and a short-day, the winged forms appeared

in either case; but not all became winged. A few remained on the snowball and produced a third and a fourth generation, the majority of which were winged. The last individual died May 26 on the snowball. It is of interest to note that the young of the third generation have a rosy tinge, and are unable for the most part to thrive on the snowball.

Eriosoma lanigera Hausman.—On February 5, a seedling infested with the apple woolly aphis was brought into the laboratory and placed in water. Winged forms were observed on April 2, and upon dissection yielded the true beakless sexes. The short days of the winter and spring months no doubt stimulated the production of the sexuparae. The winged forms were present on the bark, so that it appears that the stimulating factor is present in the sap.

Normally the fall migrants appear very sparingly during September and October, but in the laboratory they appeared in immense numbers.

THE PRODUCTION OF EGGS IN THE SUMMER

Although the great majority of the aphids lay their eggs in the fall, several species are now known to produce the sexes and eggs in summer. Gillette (*9*) finds that with *Aphis saliceti* the males and females make their appearance in June; while Doctor Patch, in a letter to the writer, states that in Maine mature oviparous females of *Macrosiphum purpurascens* were collected in June and July. In Tennessee there is an aphid on *Heuchera americana* that produces its sexes in May; and what is probably the *Georgia ulmi* of Wilson has recently been taken which deposits its sexes in May and June on a species Ulmus that is either *alata* or *racemosa*. As far as can be determined, the sexes of the latter two species are the third generation, the sequence of which appears to be predetermined, because of the small number of generations, and is not affected by the length of day.

That the length of day is not the only factor influencing the production of eggs is indicated by the fact that a few tropical aphids are known to produce eggs. Baker, in a letter to Doctor Patch, states that "in tropical countries where there is a distinct rainy and dry season and where the trees leaf twice a year, the insects seem to accommodate themselves to this condition." Das (*4*), working at Lahore, India, found that the aphids there have a definite migration and produce sexual eggs, which are normally deposited during January and February; while a few species, such as *Brachyunguis harmalae*, deposit eggs also in May that hatch in September in order to tide the insects over the dry season. Sexual forms have not been observed by Mason (*14*) in Florida, which is in about the same latitude as India. Similarly in Java, Van der Goot (*10*) states that the sexual eggs have never been found.

CONCLUSION

Sex production and migration in the Aphididae can be controlled experimentally by subjecting the hosts to definite amounts of daily light exposure and they do not appear to depend on temperature, at least in the Temperate Zones. The question naturally presents itself, whether there is a direct action of light on the insect which stimulates egg production, or whether the chemical change in the plant subjected to a short day, is the stimulating factor. Observations made in the laboratory and the fact that root-feeding aphids away from direct action of the light

produce eggs in the fall would argue for a chemical change in the sap of the host. Garner and Allard (7) have obtained correlation between the observed responses of the plant to change in the length of day and hydrogen-ion concentration of the cell sap, as well as between the content of "available" carbohydrates.

The experiments with *Aphis sorbi* would indicate that just as the short days of fall stimulate the production of fall migrants, so the lengthening days of spring stimulate the production of spring migrants to take their abode on the plantain. The length of time that this pest remains on apple or the production of an added generation is of extreme importance in several noted apple sections. To have the spring migrants produced early is what the apple grower is desirous of, and if cheap light were available he could easily do this by artificially lighting the orchard for a few weeks. Orchard heaters are now in use in many sections. Why not lighted orchards?

SUMMARY

(1) The production of the sexes of several of the Aphididae under observation has been produced experimentally in early summer and appears to be governed by the length of day to which they are exposed, regardless of the temperature.

Garner and Allard (6), who first made use of the principle of "photoperiodism," that is, the response of a plant to the relative length of day and night, were able to make ordinary fall-flowering plants blossom in summer or at any other season by the employment of a short day. Both plants and plant lice appear to respond to the same stimulus as far as sexual reproduction is concerned.

(2) The exposure of strawberry plants bearing *Aphis forbesi* to a short day of 7½ hours, beginning May 23, 1922, resulted in the production of both males and oviparous females in abundance by September 18. In 1923 strawberry plants were given the same exposure at about the time the eggs were hatching, starting February 23. The first oviparous females were observed on May 7 and the first eggs were laid May 22. The normal appearance of the sexual forms of *A. forbesi* in Tennessee is in November.

(3) Conversely, under a long exposure, beginning September 4 out of doors, the sex forms of *Aphis forbesi* were inhibited from appearing and viviparous reproduction was still taking place when examined December 3.

(4) The stimulus to migration of those plant lice having an alternate host was also found to be the relative length of day. By subjecting the secondary or summer hosts of several well-known migratory species, such as *Aphis rumicis*, *Aphis sorbi*, and *Capitophorus hippophæs*, to short days of about 7½ hours, it was possible to produce the fall migrants and males in the latter part of May and early June. Oviparous females of *A. rumicis* matured on snowball June 20, at the hottest time of the year, when the days are longest. The normal appearance of the sexes of these species is the latter part of October and November.

(5) Ten generations of *Aphis sorbi* were produced on apple by June 12, with the possibility of many more, as the result of keeping the apple seedlings in the laboratory under subdued light and giving each generation a new plant. Winged forms appeared in each generation from the third to the tenth very sparingly, with no tendency to increase.

(6) The length of day with relation to the time of hatching appears to be an important factor influencing the early or late production of migratory forms.

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